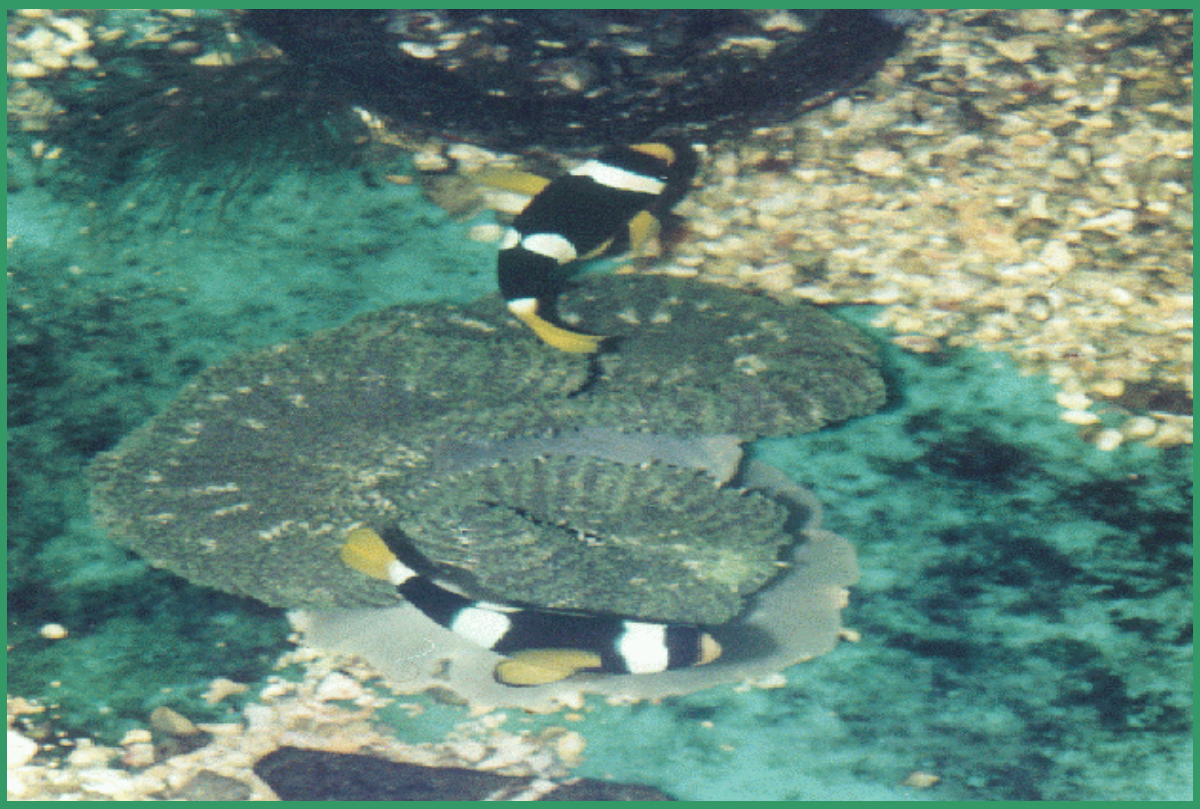
The Southern African Marine Aquarium Fish Breeder's Handbook



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CHAPTER 1: INTRODUCTION

Marine aquarium fish keeping is the largest growth area in the lucrative ornamental fish market. While overall trade in marine aquarium fish is steadily increasing, there is a growing resistance to trade in wild-caught fish, due to legislation and environmental education. It follows that if these trends continue to strengthen, the demand for farm-reared fish will be amplified. The Industrial Development Corporation of South Africa has identified the increasing demand for farm-reared marine aquarium fish as a possible area for commercial development. They funded a four year research programme at Rhodes University, to test the feasibility of rearing marine aquarium fish in a commercially sustainable manner in southern Africa.

Local demand for marine aquarium fish was estimated at R 1.5 million per annum (import parity price) in 1997. It is estimated that 60 000 marines are traded annually in South Africa, 95% of which are imported (all wild caught). Demand for marine aquarium fish is estimated to have grown by about 40% over the past 3 years and is forecasted to continue growing at 15% to 20% per annum over the next 3 years. The high growth in demand is attributed to the lifting of a 40% import duty in 1994, which resulted in the establishment of specialized marine aquarium fish shops and an increase in fish availability. The most important species bred for commercial purposes, clownfish, is estimated to represent about 16 % (R240 000) of the total value and 25 % (15 000) of fish volume.

Commercially available marine salts and trace elements make it possible to keep marine fish anywhere and demand is spread throughout the country. The main concentration of demand is located in Gauteng (60%), Western Cape (15%) and Kwazulu/Natal (10%). Based on the import statistics of ornamental fish and industry sources, there are an estimated 10 000-12 000 active marine tanks in South Africa and 0.03% of South Africans keep marine aquarium fish.

In the USA, sales were estimated at $160 million in 1995 and 0.4 % of Americans keep marines (i.e. marine aquarium fish-keeping is 13 times more popular in the USA than in South Africa). The South African market has the potential to grow, but the demographics of our society and the greater affluence of Western World countries make direct comparisons difficult.

The majority of marine aquarium fish (95%) are imported into South Africa and the major suppliers are located in Singapore, Sri Lanka, Miami USA, Kenya and the Philippines. All the local ornamental fish wholesalers and several retailers import marine fish, and some wholesalers also act as import agents for retailers who do not import fish themselves. Importers indicate that a wide choice of possible suppliers exist in each country and supplier loyalty is based firstly on price and secondly on the consistency in quality.

The imports of all ornamental fish to South Africa grew by 14% per annum (deflated at 10% p.a.) over the 5 years up to 1996 (Table 1), and were estimated to grow at a minimum of 10% per annum over the subsequent 3 years. Imports of marines are estimated to have grown by 27% per annum over the same period. Local supply (5% of demand) is very limited and erratic, with no legal commercial wild sources (local reefs are protected by marine reserves) and a limited cultured source from hobbyists and surplus stocks from the research programmes of the Department of Ichthyology & Fisheries Science at Rhodes University and the Oceanographic Research Institute (ORI) in Durban. Apart from these two research hatcheries, there are no commercial marine fish breeding facilities in southern Africa.

Table 1: Ornamental fish imports and exports in South Africa.

Year	Total Imports (R)	Marine imports (R)	Total Exports (R)
1988	452 524	54 000	180 123
1989	1 295 127	155 000	349 808
1990	1 704 871	205 000	585 729
1991	1 422 639	213 000	615 293
1992	1 361 033	225 000	879 486
1993	1 750 729	315 000	1 047 291
1994	2 399 557	480 000	955 137
1995	3 069 783	691 000	1 102 748
1996	4 415 579	1 148 000	974 519

The major threat to marine aquarium fish culture on a commercial scale is the low cost of imported wild fish. Importers land clownfish at between R13.00 to R18.00 per fish based on a cost of R3.00 to R8.00 per fish and R10.00 for transport, handling and packaging. The retailers that use import agents pay an additional 10% handling fee and the remaining retailers have to buy clownfish at R25.00 to R30.00 (100 % mark-up). Clownfish retail at prices ranging from R35.00 to R80.00, depending on the species and the geographic location of the retailer. Retailers also place a higher mark-up on the popular fish like clownfish and damselfish to subsidize the cost of more expensive fish.

Compared to Europe and the USA the local market is still unsophisticated. Captive-bred fish can be sold at a premium in these countries, due to the ecological concern of the aquarists, as well as the implementation of long-awaited environmental legislation controlling trade in marine aquarium fish in these regions. The major buying criterion for the local consumer is still low price, followed by large size and good survival.

If marine aquarium fish farming in southern Africa is to be commercially viable, it has to target the international market. The international market demands volume, variety and reliability from its suppliers. The only way to achieve this is through large-scale commercial production at dedicated marine hatcheries, or through a number of small-scale, specialist breeders who approach the international market as a co-operative unit, with a centralised collection and shipping station.

The following document is a summation of the research carried out at Rhodes University, written in manual form. It is a practical guide for aspiring commercial marine aquarium fish farmers in southern Africa, but should be equally useful for aquarists who wish to breed marine fish in their home aquaria.

CHAPTER 2: SITE SELECTION

The location of a large-scale, commercial marine aquarium fish farm depends on two important considerations; water quality, and market access. From a commercial point of view it would be best to farm marine aquarium fish in a warehouse next to an international airport. Although it is feasible to farm marine fish away from the coast, artificial seawater manufacture or seawater transport on a large scale is costly. It is also difficult to maintain good water quality in a system that has a re-use efficiency of greater than 95% per day. Paradoxically, toxins can build up in an efficient recirculating system, which can only be flushed from the system through water exchanges.

Good water quality is the most important aspect of marine fish farming, and should be of primary concern when choosing a site. The water temperature range should be close to the optimum temperature for tropical marine fish, which is 26^{o}C. This limits the ideal site selection to a very small area of the northern KwaZulu/Natal coast, near Richards Bay. By using recirculating technology, water can be heated and maintained at the correct temperature, making most areas along the southern African coastline feasible for fish culture. Unfortunately, heating requires energy, which increases the running cost of the farm, and will have an important impact on farm design and management.

The seawater supply must be free from pollutants, which limits the use of seawater near urban areas or regions of heavy industrial activity. Most of the fish produced on the farm will be for the export market, which means that access to an international airport is vital. International airports are usually located near highly populated urban areas. Fortunately there are very few areas along the southern African coast that are not within 50 km of a small airfield.

Another consideration for site selection is accessibility, in terms of infrastructure and coastal topography. Imran Klontz of the Department of Ichthyology and Fisheries Science at Rhodes University carried out a GIS-based search for suitable marine aquarium fish farm sites. He used the following site selection criteria for his study:

- Water temperature
- Land availability (Excluding urban and Nature Reserve areas)

- Land accessibility (Topography, infrastructure)
- Airfield within 50 km

He found that suitable sites exist all along the coast, if the temperature criterion is excluded (Figure 1).

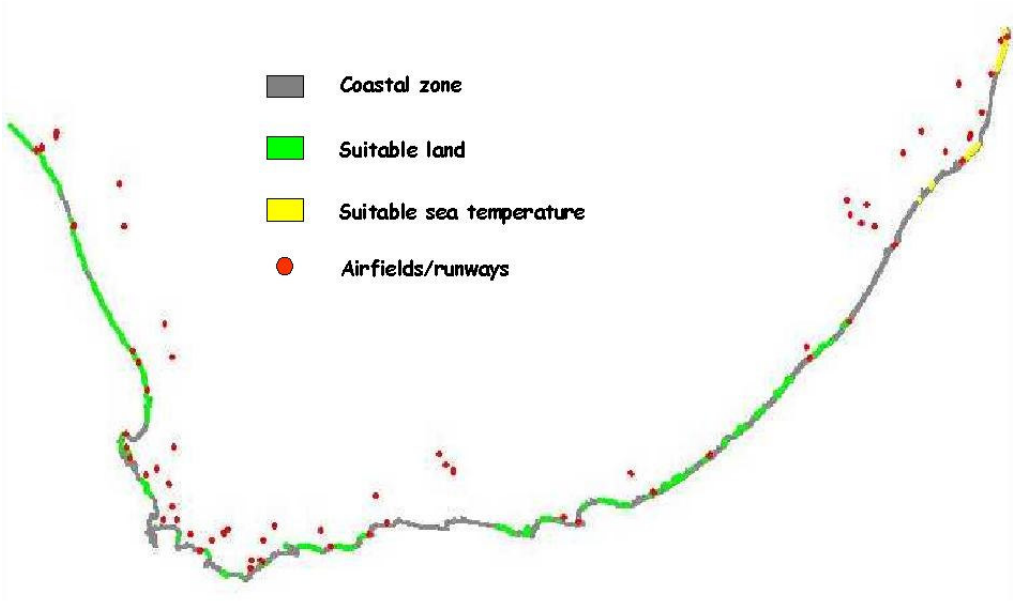

Figure 1. Possible marine aquarium hatchery sites, following the criteria outlined in the text.

CHAPTER 3: RULES AND REGULATIONS

Before procuring the use of the site, it is a good idea to sort out the legal aspect of setting up a hatchery and farming marine aquarium fish. Marine fish farming falls within the activity described as mariculture, which is now recognised as an agricultural activity rather than an industrial one by the South African government. The fishing farming operation will have to conform *inter alia*: to the following Acts:

- ✓ The Sea Fisheries Act, 1988
- ✓ The Water Act
- ✓ The Sea Shore Act
- ✓ The Environment Act

The farmer must apply to the Minister of Environmental Affairs and Tourism for the Right to undertake mariculture activities in South Africa. This Right is only available to a South African citizen, for a renewable period of 15 years. The Right may only be exercised under the authority of a permit, which will be issued annually. The permit (and Right) only relate to one site, and one category of organism, such as finfish, shellfish or seaweed etc. The application form is available from the Directorate of Marine and Coastal Management, Department of Environmental Affairs and Tourism (M&CM) in terms of sections 13 and 18 of the Marine Living Resources Act, 1998. It needs to be completed in full, and signed in the presence of a Commissioner of Oaths. A number of ancillary documents are required when applying for the Right, including:

- ✓ Proof of property ownership and/or land tenure authorisation
- ✓ Written approval from the local government authority
- ✓ Written approval from the provincial conservation authority
- ✓ Descriptions of the proposed site (including a 1:50000 map with the property clearly demarcated)
- ✓ Environmental Impact Assessment
- ✓ Details of the finances and resources available for the operation
- ✓ Description of the marketing strategy
- ✓ Details of community involvement and supporting industries
- ✓ Details of chemical and feed use
- ✓ Details of nearby activities, and their impact on the operation
- ✓ Details of water intake and discharge, extent of recirculation, effluent treatment, pipeline details

- ✓ Other documentation, as required by the Minister

The prescribed fee for an application for a Right is R100.00 at present (1/1/2000), and the fee for the renewable permit is R1000.00 per annum.

Once the Right to undertake mariculture has been granted, further permission is required to procure the brood stock and to sell the product. A permit is required to import exotic marine aquarium fish into South Africa. Application must be made to M&CM, detailing the name of the supplier, country of origin, and volume of fish. A veterinary certificate must accompany all imports. The permit costs R25.00, and must be renewed annually. Collection of local brood stock from the wild also requires permission. The local provincial conservation authority will provide the collection permit, but it is usually a once-off arrangement with the authority. Large scale or commercial collection is not permitted.

It is illegal to translocate fish species from one region to the next in South Africa, If brood stock are transported to the farm, or juvenile fish are transported to market, permission must be obtained from each of the local provincial conservation authorities through which the fish will travel.

Finally, depending on the species (Knysna seahorse, for example), an export permit may be required by M&CM.

CHAPTER 4: HATCHERY DESIGN

Once the land has been procured, it is important to choose the building site carefully. One of the greatest running costs of the hatchery will be pumping seawater ashore. Within reason, it is best to situate the hatchery as close to the shoreline as possible, to save on piping and pipe maintenance costs. Pumping against a pressure head is energetically costly, so the hatchery should be constructed as close to sea level as possible. The hatchery should be built on a flat, homogenous substrate. If the substrate is heterogeneous, it may subside and compact at different rates under the weight of the hatchery, causing cracks to appear. The floor of the hatchery must be re-enforced, to support the mass of water in the hatchery. The seawater should be pumped ashore from a well point, which is sunk into the sand below the spring low water level. It is better to pump the seawater from a well point than directly from the sea, as the sand through which the water is drawn acts as a mechanical filter.

Two important considerations influence hatchery design; energy efficiency, and disease epidemic control. It is likely that the hatchery will be built in an area where the ambient temperature rises above and falls below 26 ^{o}C as the seasons change, which means the hatchery will have to be insulated. Marine aquarium hatcheries are usually designed as one unit to optimise climate control and lower building costs. The brood stock, larval rearing and grow-out sections of the hatchery are all under one roof, within insulated walls. While this system is more energy efficient it can lead to disease management nightmares; a disease outbreak in one system can quickly spread through the rest of the hatchery. A compromise is to keep all the systems under one roof, but separate the different systems into rooms which can be effectively isolated from the rest of the hatchery. The most important system is the brood stock system; juvenile fish can always be replaced; but any loss of brood stock fish or egg production will have a disastrous effect on hatchery production.

Every hatchery should have a quarantine room. This room *must* be completely separate from the rest of the hatchery. Even if the quarantine room shares the same structure as the rest of the hatchery, it must be completely sealed off, and should only be accessed from a separate entrance, outside the hatchery. The quarantine system and all its equipment must be completely, physically isolated from the rest of the systems in the hatchery. The quarantine system should have

its own supply of fresh and marine water, air and oxygen. A well-stocked supply of pharmaceuticals should be available at all times, as well as industrial and personal disinfectants. Ideally, personnel working in the quarantine system at a large hatchery should not have access to the rest of the facility.

The quarantine room is used to keep newly acquired fish under observation for disease outbreaks, as well as to treat sick fish from the hatchery. Fish are severely stressed during handling and transport, and there is usually a 2 - 4 week lag between a stressful event and the resulting outbreak of disease. The fish need to be kept in quarantine during this time, so that the infection is not spread to the rest of the hatchery.

The basic hatchery layout is described in Figure 2. Each room can be entered from a central area, so that there is no need to pass through one room to reach another. Access to each room must be tightly controlled; only personnel working in the room should be allowed into it. Each room must be provided with electrical points and air, freshwater and seawater supply. Each system should have its own equipment, such as nets, buckets, siphon pipes, etc. Equipment must *never* be shared between systems.

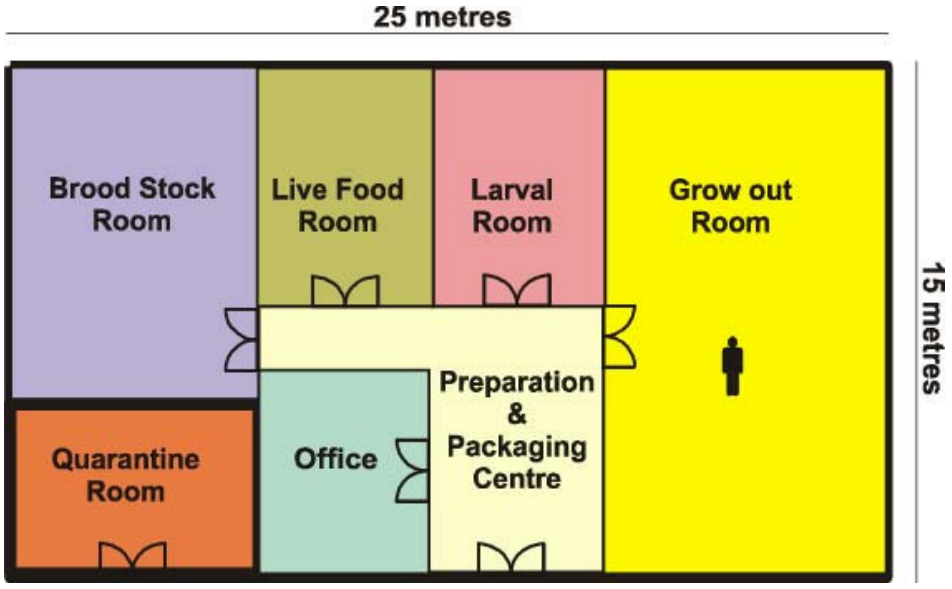

Figure 2. An example of a marine aquarium fish hatchery and grow-out facility, in one insulated building.

The outer walls of the hatchery should be insulated. This can be achieved in many ways. The outer wall of the Rhodes University hatchery consists of a double layer of bricks, separated by a narrow layer of trapped air. Many types of insulating material can be placed between a double wall in this way, but building costs will be higher, and if not carried out correctly, the building structure will be weakened. The most cost effective method of insulation is simply to cover the interior side of the outer walls with an insulating material. Many products are available that would normally be used to insulate the roof of a building, such as sisalation, polystyrene or Aerolite®. A ceiling is not necessary in the hatchery, as long as the interior of the roof is lined with insulation.

A skylight should be set into the roof of the hatchery above the brood stock room and the grow-out room (Fig. 3). Anecdotal evidence points to sunlight playing an important role in the induction of spawning in some species of fish, while the colouration of juvenile fish exposed to sunlight is more developed than in fish exposed to artificial light only. Direct sunlight may stress the fish, and promotes algal growth, so it should be diffused through a 40 - 60 % shade cloth curtain.

Figure 3. The Rhodes University marine hatchery, with the skylight above the brood stock room circled in red.

The humidity in a well-insulated hatchery containing seawater at 26 °C will quickly reach saturation point. Not only is it uncomfortable to work in these conditions, it

is impossible to maintain metal-based equipment, fittings, electrical systems, etc. for any length of time. For this reason, the hatchery must be fitted with extractor fans. Roof-mounted, wind-driven extractor fans are maintenance-free and cost effective. They should have a movable cover fitted over the air inlet at their base, so that they can be opened and closed as required.

The ambient temperature within the hatchery is maintained at a constant temperature of 21 - 25 ^{o}C by air-conditioning. Air flow through an conditioning unit is measured in British Thermal Units (BTU). Approximately 5000 BTU are required to condition a 10 m^2 room. Air conditioning units come in different sizes; wall-mounted units are rated at 24 000 BTU's, but larger external units (50 000 BTU) or roof-mounted units (150 000 BTU) are also available. At present (1/1/2000), an air conditioning unit costs about R0.26 per BTU to install. A hatchery the size of the one depicted in Figure 2 would require about 187 500 BTU; the equivalent of 8 wall-mounted units.

The water temperature in each system in the hatchery must be maintained at 26 ^{o}C by thermostatically controlled immersion heater elements. The use of heating elements in the systems allows for greater temperature control, and by keeping the ambient temperature below the water temperature, the humidity problem is alleviated. Experience has shown that only about 300 W of additional heating is required per cubic metre of seawater to achieve 26 ^{o}C in a recirculating system, if the ambient room temperature is kept above 21 ^{o}C.

The hatchery must be kept as clean as possible to discourage disease outbreaks. To this end the walls and floor of the hatchery should be coated with an epoxy paint that is resilient enough to be scrubbed clean on a regular basis. The floor of each room should tilt slightly toward a drainage point, so that spilt water does not collect on the floor. The drain should be at least 110 mm in diameter, to prevent clogging. Each room in the hatchery should be supplied with numerous, strategically located overhead electrical points. Overhead power points are used so that wiring is kept off the floor and out of the way of personnel, as well as to facilitate the spraying down of the walls and floor of the hatchery during cleaning.

CHAPTER 5: SYSTEM DESIGN

Two extremes of water use are possible in land-based marine aquaculture facilities; the flow-through system, and the re-use or recirculating system. In the flow-through system, water is pumped from the sea, mechanically filtered, sterilized with ultra violet irradiation, ozone or chemicals, and temporarily stored in large storage tanks. The water used in the fish tanks in the hatchery is drawn directly from the storage tanks. Effluent water drains from the hatchery into a water treatment plant, where it is reconditioned and allowed to flow back into the sea. This system works well in the warmer climates, where the temperature of the sea is not much lower than the required temperature of the water in the hatchery. The incoming water can be heated while in storage, but the energetic cost is prohibitive. Also, the organisms within the hatchery cannot be protected from the changes that may occur in coastal water quality (eg. Up-welling, red and black tides, pollution).

In a fully recirculating system, the seawater used in the fish tanks is pumped from a reservoir; the effluent water from the fish tanks is reconditioned using a series of mechanical and biological filters, and then returned to the reservoir. Some water exchange is unavoidable, as water lost through evaporation, spillage or leakage has to be replaced. Almost 100% water re-use has been achieved in some experimental hatcheries, using macro-algae (eg. *Caulerpa* spp.) or anaerobic bacteria filtration to remove nitrates from the system. Nitrate accumulates in a recirculating system as the final result of the nitrogenous waste breakdown process, which occurs in the biological filters. Even though algae and anaerobic bacteria can be used to remove nitrate, the process is slow and the technologies are new, with unpredictable results. Most hatcheries control nitrate build-up through a partial water exchange of 10 - 20% per week.

A compromise between the two extremes of water use is the semi-recirculating system. The system is engineered to be fully recirculating, in case of source water vagaries, but the water in the system is exchanged at a rate in excess of 10% per day, depending on energy (heating vs pumping) costs. By using this approach, excellent water quality can be maintained, without sacrificing environmental control.

The marine hatchery consists of different types of systems; for brood stock,

larval rearing, juvenile grow-out, quarantine and live food culture. Each system consists of four facets; tanks, biological filtration, mechanical filtration and sterilisation. Each facet can be made up of a number of parts, depending on the requirements of the system. Excellent descriptions of the different types of tanks and filters used in aquaculture, as well as discourse on their pros and cons, may be found in works by authors such as Fred Wheaton[1] and Stephen Spotte[2].

5.1 Brood stock system.

The size, shape and depth of the brood stock tanks are dependent on the species. Most of the tropical reef species that can be bred in captivity do not require large or deep tanks. Clownfish for example, seldom forage far from their nests, requiring relatively small, shallow tanks of approximately 80-100 ℓ. Some species of seahorse, on the other hand, require tanks deeper than 40 cm, so that they may complete their complex vertical mating dances.

The tanks can be made of many different types of material, including glass, fibreglass, PVC plastic, fibreglass-coated marine ply-wood; even concrete. The prerequisite for the construction material is that the inner surface of the tank is smooth, watertight and stable in seawater. This is particularly important when using PVC plastics. Heavy metals such as cadmium are sometimes used as a colouring pigment, which leaches out of the plastic in time, and can poison the organisms in the tank. At least one side panel of the tank should consist of glass or clear perspex, so that the behaviour of the fish can be observed. Tank shape is usually rectangular, primarily for economy of space. All tanks should be covered with sturdy lids, as marine aquarium fish tend to jump when excited.

The bottom of the tanks should be covered with some form of substrate, and the fish must be supplied with a refuge. All tropical marine species require some form of refuge in the tank, to take the place of the refuges provided by the coral and reefs of their natural environment. The type of substrate and refuge depends on the species. Clownfish, for example, only require a shallow substrate of gravel, or crushed coral or shell, which they rearrange during breeding rituals. An earthenware flowerpot lying on its side is an acceptable alternative to a sea anemone for a refuge, and provides an excellent surface upon which to lay eggs.

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[1] Wheaton, F.W. 1977. *Aquacultural engineering*. John Wiley & Sons, Inc.

[2] Spotte, S. 1992. *Captive Seawater Fishes: Science and technology*. John Wiley & Sons, Inc.

The blue-cheek gobies (*Valenciennea strigata*) on the other hand, require a relatively deep (>50 mm) substrate of crushed coral, which they bite into and sift through their gillrakers, feeding on the small organisms they filter out in this way. An inverted flowerpot provides them with an ideal cave refuge, in which they lay their eggs.

The inflow water is added to the tanks at the surface, at a rate of approximately one exchange per hour. Water is drained from the bottom of the tanks, using either the upstand pipe or drop pipe method (Fig. 4 a&b). The outflow pipe should be twice the diameter of the inflow pipe. This practice applies to all the recirculating systems in the hatchery; all drainage pipes should be twice the diameter of the inflow pipes leading from the pump. This ensures that water cannot enter a tank faster than it can leave it, causing flooding.

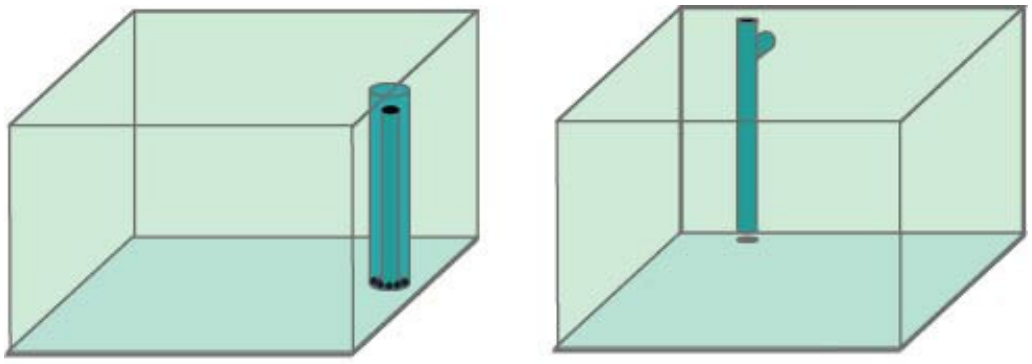


Figure 4. Drainage systems that are used in the brood stock and grow-out tanks.

The tanks can be set up as stand-alone systems, using an under-gravel, plenum, internal cartridge or external biological filter, or as part of a large recirculating system. The advantage of the stand-alone tanks is that each pair of brood fish is isolated, in the advent of a disease outbreak. However, there are many drawbacks to stand-alone systems. They are energy inefficient, as the temperature of each tank needs to be regulated. They are labour intensive, as the filters need to be regularly maintained, the salinity of each tank has to be monitored and the water in the tanks need to be constantly topped up. Also, the micro-environment of each tank is different, and there is no water quality buffer effect which one has with the larger, recirculating system. Under-gravel and plenum filters are difficult to

maintain in clownfish tanks, as the fish continuously rearrange the substrate as part of their breeding behaviour.

There are many possible filter configurations available for the brood stock recirculating system. Each filter configuration should perform three primary functions; the removal of metabolic waste through biological filtration, the removal of particulate waste through mechanical filtration, and the removal of dissolved organic waste through foam fractionation.

The first configuration is based on the serial, submerged-medium filter. This filter consists of a bed of graded stone medium, where the effluent from the tanks is channelled through a sequence of increasingly smaller bacteria-laden granite chips. The graded stone acts as a mechanical filter, and the bacteria colonising the surface of the stone reduce ammonia to nitrite, and nitrite to nitrate (nitrification). This type of bio-filter is usually connected in series with the recirculating system, which means that all the water in the system is passed from tank, to reservoir, to filter in one exchange loop. The longer that the effluent water is exposed to the stone medium during its passage through the filter, the more efficient the filter. This is why the filter usually consists of a series of baffles containing the different sized media (Fig. 5). The same effect can be attained by passing the water through a series of small, false-bottomed tanks of graded stone. Water is drawn from beneath the false bottom and is passed to the surface of the next tank. The draw back of this type of filter is that its mechanical filtration capability makes it a high maintenance, inefficient biological filter.

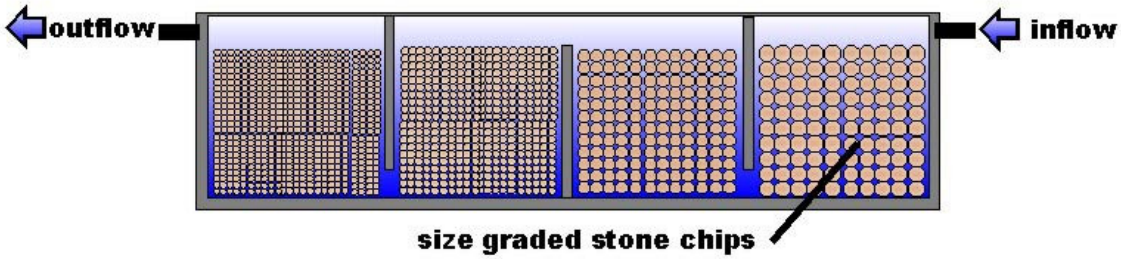


Figure 5. Submerged, graded medium filter bed.

The bacteria in a biological filter colonises the exposed surface of the filter medium in the water; the more exposed surface area, the more bacteria, and thus

the more efficient the biological filter. However, as the bacteria reproduce and die, an expanding bacterial mat forms, reducing the efficiency of the surviving bacteria on the exposed surface area. If the interstitial spaces between the filter media are clogged with particulate matter, the exposed surface area of the medium is drastically reduced. Flow rate through the filter is reduced, and canalisation can occur, allowing large portions of the filter to stagnate and die. These problems can be overcome to some extent if the water is mechanically filtered before it enters the serial, submerged medium filter. However, if the water is going to be mechanically filtered anyway, there are far more efficient biological filters available.

The most common filter configuration in use at present is the exposed medium trickle tower, also known as the drip or wet/dry filter, coupled to a sand filter and a foam fractionator. The trickle tower is cheap and easy to construct, and is very low maintenance. It does not get clogged with waste matter, and excessive bacterial mat build-up periodically "sloughs off" the filter medium through a combination of gravity and the mechanical action of the flowing water. The filter consists of a column of light-weight plastic medium which has been designed to have a high surface:volume ratio. Commercial products such as Bio-balls, Bio-strata™ and Bio-pak™ are available, but other light-weight, inert media can also be used, such as hair curlers, or shredded plastic strips, which have a surface:volume ratio greater than 250 $m^2.m^{-3}$. The filter medium column is packed within a water proof container, such as a plastic barrel, canvas bag, or large diameter (200 mm) PVC piping. The ideal shape for the tower is tall and round in cross-section, so that no dead spaces occur within the medium, and the water has a longer retention time in the filter (Fig. 6).

The trickle tower filter is usually connected in parallel to the primary water exchange loop. Some of the water is diverted before it is pumped to the tanks, and is sprayed evenly over the top of the media in the tower using a diffuser such as a shower rose, spray bar or rotary sprinkler. The water trickles down through the tower, coming into contact with the bacteria colonising the damp, exposed media. The thin film of water passing over the media becomes highly oxygenated, allowing the aerobic nitrifying bacteria to function optimally. Water flow through the filter must be fast enough to ensure that the media is kept damp, but slow enough to prevent the sloughing off of the bacteria due to mechanical action, or to flood the filter. The treated water then passes out of the open-ended tower and is collected

in a sump or the reservoir of the system. The trickle tower also acts as an aeration and degassing column. The thin film of water covering the media has a large surface area, allowing the supersaturated gases in the water to escape, whilst becoming saturated with oxygen. Super-saturation occurs when air and water combine under pressure, and gases in the air are forced into solution, even though the water is already fully saturated with the gas. This sometimes happens if air gets into the impeller chamber of the pump, or is entrained into a high-pressure pipe through an air leak. Super-saturated water can harm the fish, causing gas bubble disease.

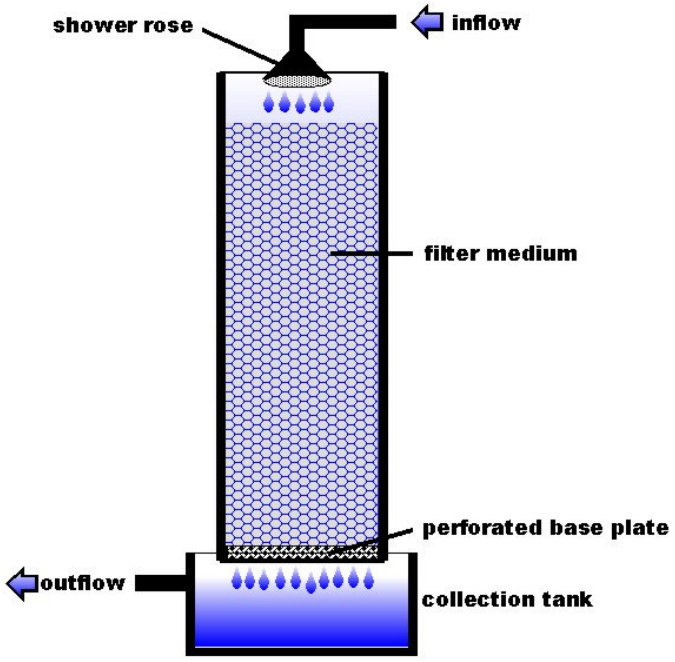


Figure 6. Exposed medium trickle tower.

The effluent water from the tanks drains into the large sump or reservoir of the system. Water is pumped from the sump through a pressurised sand filter, which acts as a mechanical filter and traps particulate matter larger than 100 μm. Although industrial marine water pumps and sand filters are available, they are expensive. Swimming pool pumps and sand filters are just as effective for a medium sized recirculating system and are cheap and readily available. Sand filters use up a sizable portion of the pressure head developed by a pump, and this must be considered when designing a system. A combination swimming pool pump and sand filter can be bought as a unit (Fig. 7), and their rate of water delivery is

measured post-sand filter, making it easier to purchase the correct combination for your system. It is best to over-estimate your water requirements, as sand filters clog quickly. This increases the water pressure needed to pass through the sand filter, and less pressure is available to the rest of the system. Sand filters should be back-flushed daily, to wash the collected matter from the filter, and to resettle the sand to prevent canalisation. At the Rhodes University marine hatchery, 0.5 kW swimming pool pumps coupled to sand filters were used to supply 4.0 m^3 recirculating systems with ample water flow. There is enough pressure to pump water in a 160 mm diameter pipe to a head in excess of 2.0 m.

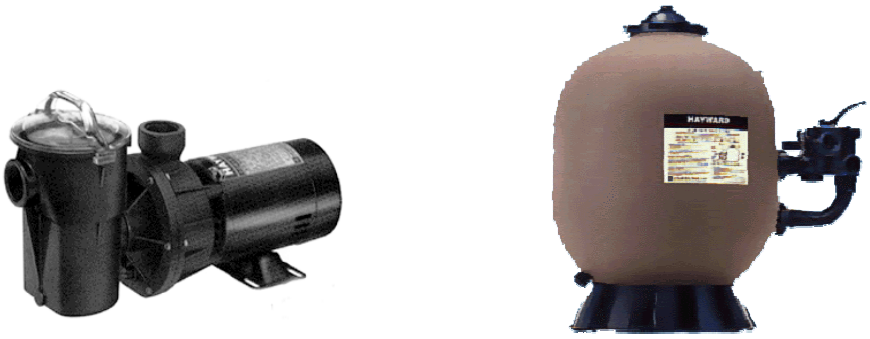


Figure 7. Typical swimming pool pump and sand filter that can be used to drive the recirculating systems of a marine aquarium fish hatchery.

After mechanical filtration the water is passed through a foam fractionator, also known as a protein skimmer or air stripper. There are many commercially available fractionators, but they are very expensive. Excellent fractionators can be made very cheaply. There are many designs available, but they all perform the same function, to a more or less efficient degree. A fine mist of air bubbles is passed through a column of water in the contact chamber. Electrically charged organic molecules concentrate at the air-water interface of the bubble, with their charged (hydrophilic) ends pointing into the seawater, and their non-charged (hydrophobic) ends protruding into the air. In this way they develop a thin film of surface-active material around the air bubbles, which burst on surfacing, forming layers of foam. The foam is collected from the surface of the water, effectively removing both dissolved and particulate organic waste from the system.

The ideal bubble size should be approximately 0.8 mm in diameter, introduced into the contact chamber at a rate of 1.8 $cm.sec^{-1}$ per square centimetre of chamber cross-section (Spotte, 1992). Bubbles are usually produced through stone or

wooden diffusers, or by the venturi effect of air entrained into fast flowing water. Water in the contact chamber may flow with the rising bubbles, or, more effectively, against the flow of the rising bubbles (countercurrent system). A simple countercurrent foam fractionator design is outlined in Figure 8. The height of the contact chamber depends on the air pressure (or pump pressure in the case of the venturi system) available. The foam fractionator is connected to the main recirculating system in parallel, after the water has passed through the mechanical filters, and drains back into the reservoir of the system.

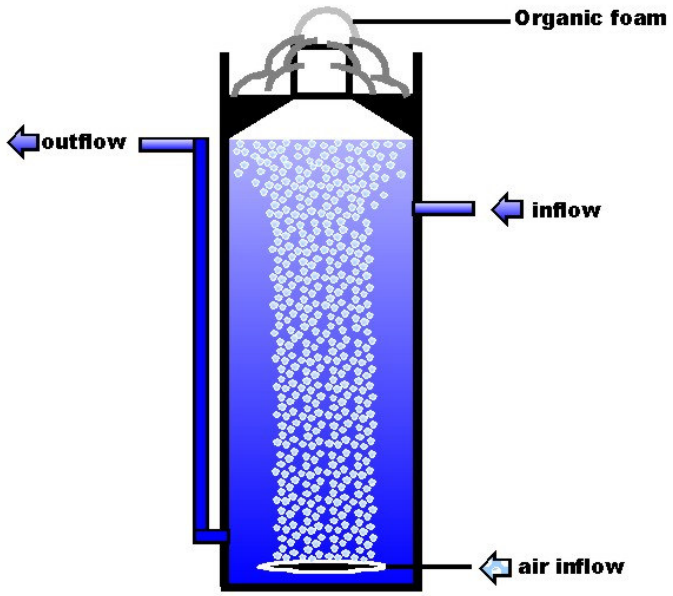


Figure 8. A simple counter-current protein foam fractionator.

The third configuration is basically the same as the second, but a fluidised bed up-welling biological filter replaces, or complements the trickle tower bio-filter. The up-welling filter consists of a tall tower usually constructed from a large diameter (200 mm) PVC or acrylic pipe. The tower is half-filled with small, rounded filter granules, such as course river sand, glass beads or high-density plastic beads. Water is pumped into the bottom of the tower, through a diffusion plate, under pressure, causing the granules to separate and "fluidise" in the water column (Fig. 9). The water pressure should be powerful enough to lift and separate the granules, without forcing them out of the top of the tower, with the effluent water. The up-welling tower is the most efficient of the biological filters, as the entire surface area of each of the granules can be colonised by bacteria, and is

exposed to the waste-carrying water. The fluidised nature of the filter bed means that it cannot become clogged with particulate matter, while the tumbling action and continual collisions of the granules ensure that excessive bacterial mat build up is removed. The only draw back of the up-welling filter is that it requires a substantial amount of constant-head water pressure to keep the filter granules fluidised. The up-welling filter is usually supplied by a separate water pump, drawing water directly from the reservoir, and either draining directly back into the reservoir, or via a trickle tower. Some oxygen depletion occurs in the up-welling tower, which is why it is usually used in conjunction with a trickle tower.

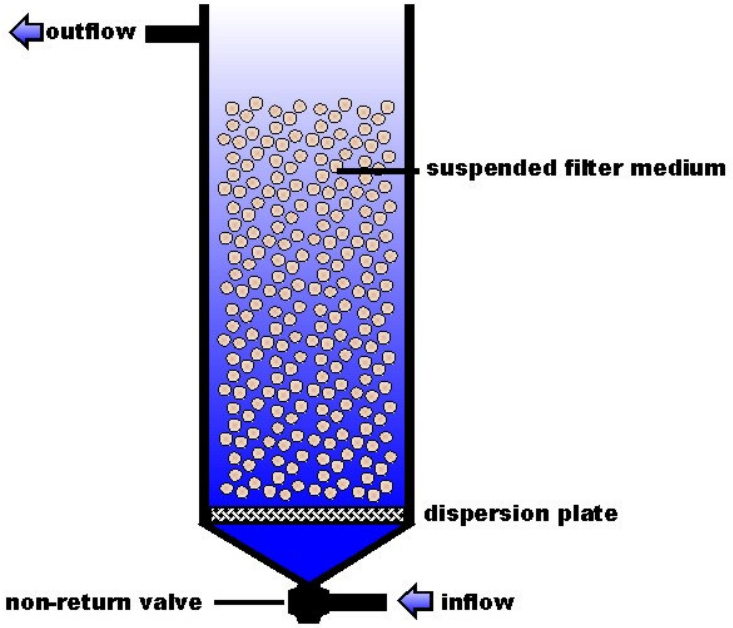


Figure 9. A fluidised-bed upwelling biological filter.

The bio-load per brood stock tank is normally very low, consisting of a pair of fish, seldom exceeding 20 g each. The brood stock fish are hand-fed a range of diets to visual satiation twice daily. Consumption is carefully controlled, the emphasis being on quality, to maintain breeding condition, rather than quantity, to promote growth, as in the grow-out system. This means that there is little demand placed on the filters due to excess, uneaten food. The amount of biological filtration required by a recirculating system depends on the mass of fish supported by the system, and the amount of food fed to the fish.

The volume of the bio-filter can be calculated according to the method described below. Although is almost impossible to accurately calculate the required biological filter volume for a commercial recirculating system, we can try for an estimation that should at least be in the right "Ball Park".

Food, in the form of protein, is the primary source of nitrogen metabolites, whether processed by the fish and converted to ammonia and urea, or left in the system to break down through bacterial action. The most toxic product of protein breakdown is ammonia. Ammonia exists in two phases when in solution, NH_4^+ and NH_3. Because NH_4^+ is ionic, it normally exists as part of a larger, complex molecule. It is too large to pass through the epithelial membrane of the fish, and is therefore less toxic than NH_3. The un-ionised NH_3 molecule is able to enter the fish, concentrating in the blood and poisoning the fish. The two phases of ammonia exist in a state of equilibrium, where the relative concentration of the NH_3 phase increases with increasing pH and temperature.

Aerobic bacteria break down the ammonia to nitrite in the biological filter. Nitrite is also toxic to the fish at similar concentrations to ammonia, but in a properly functioning biological filter the nitrite is broken down at the same rate as the ammonia. The product of the denitrifying bacteria is nitrate, the last stage of the nitrogen metabolite breakdown process. Nitrate is a stable and relatively inactive molecule that is 100 times less toxic than ammonia. Even so, nitrate concentrations can build up to a level that is toxic to the fish. Nitrate is normally flushed from the system during water changes, but can be broken down very slowly by anaerobic bacteria.

The recirculating system is a closed one, thus we can assume that the amount of nitrogen put into the system as food has to be balanced by the amount nitrogen converted to nitrate. The nitrifying and denitrifying bacteria appear to be fairly consistent in the amount of nitrogen metabolite they can process in a given time, for a specific temperature, pH and salinity. They convert approximately 0.30 g ammonia to nitrate per square metre of covered surface area per day in seawater at a temperature of 26 °C. The bacteria form a monolayer on all the surfaces exposed to the ammonia-bearing water in the biological filter as well as the tanks and piping of the system itself. In essence a biological filter is just an area within the system loop that contains a material that has a very high substrate surface area to volume ratio. Each type of substrate has a fixed surface area:volume ratio

(Table 2).

Table 2. The surface area to volume of some biological filter substrates.

SUBSTRATE	SURFACE AREA:VOLUME
Earthenware balls (1.0 cm dia.)	270 m^2/m^3
Hair curlers (6.0 cm)	260 m^2/m^3
Honeycomb tube (2.5 cm cells)	130 m^2/m^3
Honeycomb tube (4.0 cm cells)	1000 m^2/m^3
Honeycomb tube (8.0 cm cells)	500 m^2/m^3
Styrofoam packing 'peanuts'	225 m^2/m^3
5 cm pieces of 50 mm pipe	124 m^2/m^3
PVC plastic netting	350 m^2/m^3
Plastic packing fibres	1440 m^2/m^3

If the amount of exposed surface area in the system is known, then the amount of ammonia that can be processed by the bacteria can be calculated. By the same token, if the amount of ammonia in the system is known, then the substrate surface area required in the biological filter can be calculated, and thus the volume of the filter unit. The ammonia input into the system depends on the amount of food fed per day, and the protein content of the food.

For example: Estimate the size of a biological filter unit required to support a clownfish brood stock system.

Variables:

Average mass of fish	=	10 g
Number of fish	=	1000
% food fed/day	=	8.0 %
Protein in food	=	40 %
Protein utilization by fish	=	60 %
% Nitrogen in protein	=	16 %

Bacteria nitrification rate	=	0.3 g/m^2
Surface area of substrate	=	260 m^2/m^3 (hair curlers)

Calculations:

Food fed/day	=	mass x No. x % food/body weight
	=	10 x 1000 x 0.08
	=	800 g
Nitrogen/day	=	food/day x % *P* in food x % unused *P* x % N
	=	800 x 0.4 x 0.4 x 0.16
	=	20.5 g N/day

∴ Ammonia input into system is about 25 g NH_3/day.

Ammonia conversion rate	=	nitrification rate x surface area
	=	0.3 x 260
	=	78 g/m^3/day

∴ NH_3 conversion rate in system is 78 *g per cubic metre of filter substrate per day.*

Volume of bio-filter required	=	ammonia input/ammonia conversion
	=	25/78
	=	**0.32 m^3**

Having calculated the volume of the biofilter following the above procedure, it must be re-iterated that the final result is an estimate at best. I recommend that a fudge factor of at least 50 % be added to the calculated filter volume. After all, it is better to overestimate the size of the biological filter, than have one that is too small.

The brood stock system does not need large or complex filter systems, but it does need stable water quality. This can be achieved by having a large sump or reservoir relative to the size of the tanks. Ideally, the reservoir should contain the same amount of water as all the tanks put together. This large body of water acts as a buffer to slow down chemical or physical changes in the water. By placing a series

of baffles in the reservoir, the flow rate of the water passing through the reservoir can be slowed, allowing larger particulate matter to settle out of the water, where it can be siphoned from the reservoir. The reservoir should be insulated to buffer temperature changes, covered with a lid to prevent evaporation, and kept dark, to inhibit algal growth. While algae may remove some of the nitrates in the system, it has a more pronounced effect on water quality, influencing pH, carbon dioxide, and oxygen levels diurnally, as well as clogging filters with organic matter.

Constant temperature control is vital for stable water quality and for maintaining brood stock condition. As previously noted, the Rhodes University hatchery uses an antagonistic system to maintain constant temperature. While the ambient temperature of the brood stock room is kept within a range of 21-25 ^{o}C through air-conditioning, an immersion heater element is used to heat the water in the recirculating system to a constant 26 ^{o}C. This system allows for very accurate temperature control.

5.2 Grow-out system.

The design concept of the grow-out system is different to that of the brood stock system. The grow-out system consists of large tanks (500 – 1000 ℓ) containing relatively fast-growing juvenile fish at high densities. The tanks contain no substrate or refuges. Water is added to the surface of the tanks at a rate of approximately two complete exchanges per hour, in a counter clockwise direction (in the southern hemisphere), so that the water in the tank is constantly circulating horizontally. A number of simple airlift pumps, affixed to the sides of the tank, are used to aerate the tank, and circulate the water from the bottom of the tank to the top. Attaching a directional nozzle to each airlift pump, and pointing them in the direction of the surface flow can further increase the velocity of the horizontally moving water in the tank. The vertical and horizontal movement of the water ensures that no "dead spaces" of poor water quality occur within the tank, and that particulate waste does not accumulate on the bottom of the tank. The particulate waste is flushed from the tank through the upstand pipe or drop pipe, with the effluent water. Circular grow-out tanks would be ideal, even though they are not space efficient. They should have a conical base, with the drainage point located in the centre of the tank. The self-cleaning efficiency of circular tanks may be worth the increase in the grow-out area required.

The objective is to maximise the growth and survival of the juvenile fish in the grow-out system, which means a more intense feeding regime, and a subsequently higher metabolic, organic and particulate waste load than in the brood stock system. The relatively high density means that the fish are susceptible to behavioural stress, which is exacerbated by the lack of water quality buffering through the high fish-to-water-volume ratio. The fish are susceptible to disease, which can proliferate to epidemic proportions in the detritus of unclean tanks. For these reasons, the filter unit is larger and more complex than for the brood stock system, with the emphasis on mechanical filtration.

The baffles in the grow-out system's reservoir will cause the larger waste particles to settle out of suspension, where they must be siphoned or drained off on a daily basis. The sand filter will remove suspended particles above 100 μm, which must be back-flushed every day. Additional mechanical filtration is required, using either high-pressure cartridge filters, or bag filters. High-pressure cartridge filters are commercially available high volume, in-line filters that can remove particulate matter as small as 5 μm from the water (Fig. 10). They are fitted in series after the sand filter, so that all of the water pumped from the reservoir passes through the high-pressure filter before reaching the bio-filters, foam fractionator and tanks. Unfortunately, these filters use up a large amount of the water pressure available to the system, clog up quickly, are difficult to clean, and the filter cartridges are short-lived and expensive to replace.

Figure 10. An example of a commercially available high-pressure 5 μm cartridge filter.

Bag filters, on the other hand, are low-pressure filters that are connected in parallel to the system. Some of the water that has passed through the sand filter is diverted back to the reservoir via the bag filter, which constantly removes particles larger than 50 μm from the system (Fig. 11). This type of filter requires no extra pump pressure, is easily cleaned once a day, and the filter bags are re-useable.

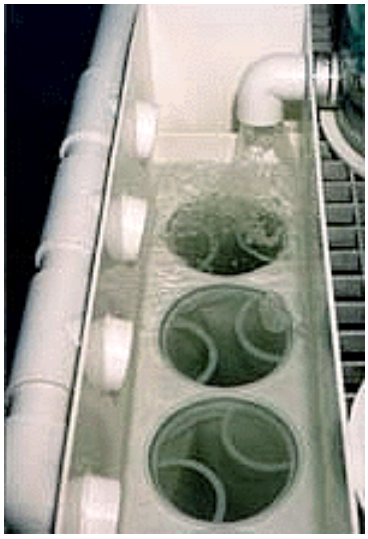


Figure 11. A number of low pressure, 50 μm bag filters.

After mechanical filtration the water is diverted to the foam fractionator and the combination upwelling and trickle tower bio-filter unit, while the rest of the water is pumped back to the tanks. Pumping water directly into the tanks under pressure could lead to gas super-saturation, and variations in flow rate as the sand filter or other pressure-absorbing filters clog up. It is best to allow the water to drain into the tanks by the force of gravity alone. This can be accomplished by pumping the water to an overhead reservoir, or "header tank", which drains into the tanks. Unfortunately this system is not space efficient, and the primary pump cannot be run continuously without a complex system of mechanical or electronic gadgetry to ensure that the header tank does not overflow, or drain completely. In a marine system, a pump that is allowed to run continuously lasts far longer than one that runs at intervals. Also, the continuous draining and refilling of the header tank causes fluctuations in the flow rate of the water draining into the tanks. The Rhodes University hatchery uses the far more efficient open U-tube system. The tanks are supplied with water from a large (110 mm diameter) "U"-shaped tube

made from PVC piping. Mechanically filtered water from the primary pump system is allowed to splash into the open end of one of the 1.0 m high uprights of the U-tube. At the other end, the excess water overflows out of a drainage point about three quarters of the way along the upright and drains back into the reservoir of the system (Fig. 12). In this way, a constant pressure head of approximately 0.75 m is maintained in the horizontal section of the U-tube, from which the water flowing to the tanks is drawn.

Figure 12. The U-tube "constant head" inflow system used at the Rhodes University hatchery.

5.3 Larval system.

In the larval system, the emphasis is on water quality. A larval tank should hold a volume of water large enough to buffer changes in water quality, but be small enough to maintain the correct live food densities economically. Round tanks holding approximately 100 to 200 ℓ are ideal, and should have shallow conical bottoms, with a sunken drainage point at the base (Fig. 13). Drainage occurs through a removable, 100 μm or 350 μm sieve which fits snugly into the sunken base of the tanks. They be constructed out of any material that is stable in seawater, but preferably fibreglass. The sides of the tank must be a dark matt in colouration, but a light coloured bottom will make it easier to keep the tank clean, and to see the larvae. The dark, matt-shaded sides of the tank help to lessen the occurrence of "head butting" syndrome. This occurs when the larvae are attracted

to the light reflected from the interface of the water with the sides of the tank. Instead of dispersing evenly throughout the water column and feeding, the larvae congregate at, and swim into the sides of the tank.

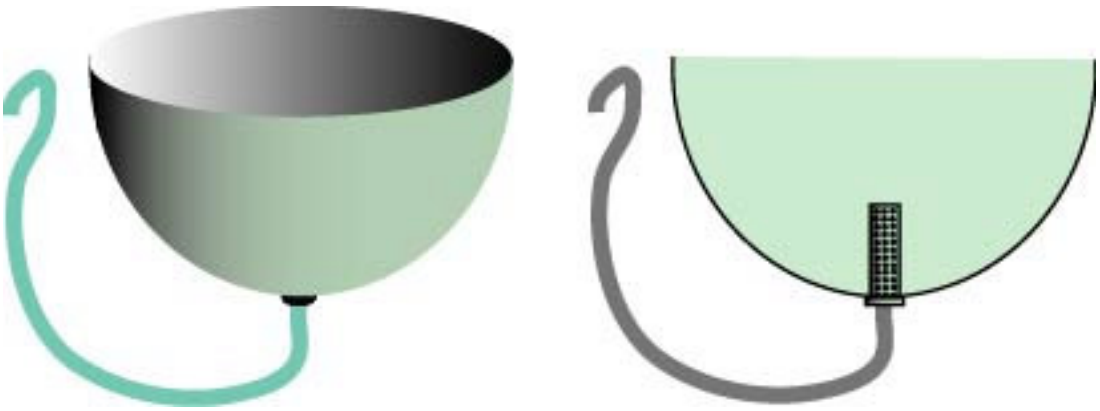


Figure 13. Larval tank and schematic depicting the central drainage sieve.

The effluent water from the tank drains out through a flexible hose. The water depth in the tank can be varied by adjusting the height of the flexible effluent pipe. The water passes through a 50 μm bag filter before flowing into the reservoir, which contains at least the same water volume as the sum of all the larval tanks in the system. The water in the reservoir is constantly cycled through an upwelling, fluidised bed biological filter column and a venturi protein skimmer. The water is pumped through a 5 μm cartridge filter and then allowed to flow through a water sterilization unit.

The water is sterilized with ozone or ultra-violet light radiation. Ozone (O_3) is manufactured by passing a powerful electric current through oxygen, using a commercially available ozone generator. A good ozone generator is expensive, and ozone is a caustic substance with which to work. The low volumes of water recirculated in the larval system makes UV radiation the preferred means of sterilization. A well-designed UV sterilization unit is cheaper to run and requires less maintenance than an ozone generator. Greater than 80 % of the bacteria in the water can be eradicated per recirculation, if the film of water exposed to the UV light is shallow, and the water is clear of particulate matter. The high energy, short wavelength light is absorbed and scattered quickly, and cannot penetrate very far into water. A simple UV sterilization unit consists of a bed of UV light

tubes below a thin layer of glass (preferably quartz), set at an angle of about 80° (Fig. 14). Recirculated, mechanically filtered water is added to the unit at the highest end, and trickles down the irradiated glass plate in a film. The sterilized water is collected at the lower end, and is pumped to the tanks. The UV radiation is harmful to other living organisms, so the whole unit should be placed within a sealed light box. The UV light tubes must be replaced every six months, as the quality of UV light emitted by the tubes deteriorates over time.

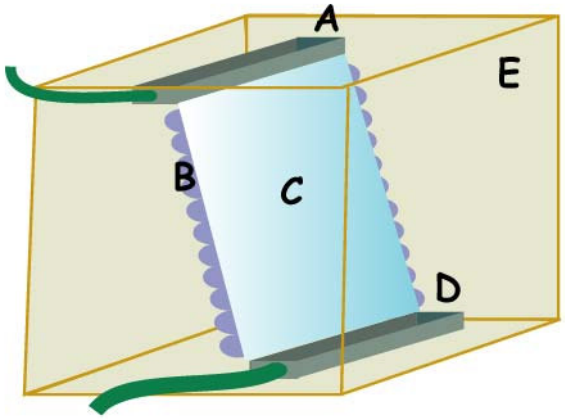


Figure 14. *A* simple ultra-violet light sterilization unit, consisting of an inflow trough (*A*), UV light tubes (B), quartz plate (*C*), outflow trough (D) and light box (E).

The purified water is pumped through a small scale U-tube type "constant head" system made from 50 mm UV-stable PVC piping. Water is drawn from 5 mm Teflon® flow control taps inserted into the U-tube, and flows into the larval tanks through detachable clear plastic tubing.

The larval tanks are illuminated by metal halide lamps set to produce an intensity of 500 Lux at the water surface. The lights are controlled by an automatic timer switch, set to a photoperiod of 12 hours of light and 12 hours of dark. An overhead "Blue Moon" night-light is set to run on a counter-photoperiod, so that the larval system is never in complete darkness. Larvae tend to sink to rest on the bottom of the tanks at night and are susceptible to bacterial infection. However, in the presence of partial light they float in the water column just above the bottom.

CHAPTER 6: HUSBANDRY

6.1 Brood stock pair formation

It is best to try to collect your brood stock fish from the wild as pairs. About 90 % of these pairs will be maintained in captivity, if the fish are handled carefully. Unpaired wild caught or hatchery reared fish have to be induced to form pairs. This is often one of the most time consuming and frustrating aspects of marine fish breeding. Pair formation is usually accomplished in large mono-species community tanks, which contain plenty of refuges. Dominant fish will take up residence in the refuges. If the conditions are right, the fish in the tank will mature and begin to form partnerships. This can take many different forms. In the clownfish species, the large dominant female in the tank will aggressively harass a smaller fish, until it takes on the characteristics of a male, and moves into the refuge with the female. The clownfish pair for life. If a partner is lost, it takes a clownfish about 30 – 40 days to recover, and start looking for a new mate. The bluestreak gobies are easier to pair up, as they do not share the same level of pair bonding (Fig. 15). If a partner is lost, they will take on a new one almost immediately. Some species are more difficult to pair up. The pygmy angelfish are aggressive to other members of their species, and may take the best part of a year to develop pairs. The most difficult species of all the fish kept at the Rhodes University hatchery were the dottybacks. These solitary fish are extremely aggressive to all other fish, and will fight members of their own species to the death.

Figure 15. The bluestreak goby, *Valenciennea strigata*.

An important thing to remember is to never introduce a new brood stock fish into an established pair-forming community tank. The established fish will attack the new fish, and the new fish will disrupt the "negotiations" already underway in the tank.

6.2 Brood stock conditioning

Once pair formation occurs, the new pair is removed from the community tank to an isolated brood stock tank. The water conditions in the brood stock tank must be kept very stable, at the optimum environmental conditions for the species. A stable temperature (26 °C) and salinity (35 ‰) is vital for all species, and photoperiod and light intensity can also be very important for some species (14Light:10Dark and 8×10^{23} quanta.sec^{-1} for clownfish). Natural light is very important for the brood stock fish, and the brood stock room should have large windows or skylights. Natural photoperiod and light intensity should be augmented with metal halide lamps, as they produce the broadest spectrum of light.

Ammonia and nitrite levels should not increase above 0.1 mg/ℓ and nitrate must not exceed 50 mg/ℓ. Oxygen concentration must be kept above 80% saturation. There should be a constant, gentle movement of water in the tanks, to ensure that no dead spaces occur where poor water quality conditions can develop. The tanks must be kept as clean as possible, without disturbing the fish too much. Algal accumulation on the surfaces in the tank should be removed once per week, while other accumulated debris and uneaten food should be siphoned from the tanks every morning and evening. The brood stock tanks must be kept in an isolated room. Only brood stock personnel should have access to this room. The brood stock must be kept under constant observation, as behavioral changes are a vital part of monitoring the health of the fish, and their readiness to lay eggs.

Fish courtship behaviour is very specific. Clownfish demonstrate their readiness to form a pair by chasing behaviour. They then settle down in a refuge as a pair, and begin to clean specific surfaces in the tank. They also rearrange the tank substrate. The male often "wiggles" in front of his larger, female partner, in a submissive gesture reminiscent of sperm release. As the courtship gets more intense, one surface area in the tank is chosen and both fish clean it vigorously. Meanwhile the female begins to thicken in the abdominal region as the eggs in the ovaries develop. Both fish display aggression to any stimulus outside the tank. The

"angle of attack" at which the female cleans the chosen surface area increases as egg laying draws near. On the day that she lays the eggs she approaches the surface at an angle beyond the perpendicular. This almost "upside-down" behaviour is very characteristic. Egg laying usually takes place in the afternoon, but has been recorded at almost any time of day. Egg laying takes anywhere from 10 minutes to an hour. The female lays the eggs in ever widening circles, with the male following in behind to fertilize the eggs. As many as 200 to 2000 eggs are laid per batch, depending on the species, size and maturity of the female. Egg size varies according to species, but is usually in the region of 1.0 – 1.5 mm in length. The eggs are guarded and meticulously looked after by both parents, but primarily by the male. Dead or deformed eggs and debris are continuously removed from the batch, and the eggs are gently fanned to maintain high levels of oxygen. Egg care continues throughout the night. After they have just been laid, healthy eggs appear bright orange in colour, from the colour of the yolk. As they develop the eggs change colour from orange to grey, then black. The day before hatching the eggs appear silvery, with the metallic blue/green pigment of the eyes of the larvae showing through. On the day of hatching, the reflective integument of the larvae also shines through the egg. The eggs hatch about 45 minutes after sunset (or lights out). Courtship behaviour begins the following day, and a new batch of eggs are laid within 2 days to a week.

Another example of fish courtship and egg laying is the bluestreak goby. In this species, the sexes are is distinguishable by the second dorsal ray of the male, which is twice as long as that of the female. The pair are usually inseparable, swimming and feeding side by side in the tank. The female develops a reverse "C" shaped discoloration anterior to her anal vent when she is ready to breed, which may mimic the swelling of her lower abdomen, which occurs as her ovaries mature. The swelling is distinctive, and is accompanied by the male gently butting at the region of her anal vent. Both fish will disappear into their refuge in the afternoon of the day of egg laying. Egg-laying and fertilization takes place in the refuge. The male will then emerge and seal the female in the refuge with the eggs. The tiny eggs are laid in a gelatinous clump attached to the roof of the refuge. After three days the eggs hatch under the cover of darkness. The gobies are ready to breed again within a week.

The brood fish are fed twice per day, in the morning and early evening. Three basic food types are fed, on a rotational basis; a wet diet, a dry diet (usually flakes) and

black mussels (*Perna perna*). The mussels are an excellent diet for the production of good quality eggs. The dry flake food must be obtained from a reliable brand such as Wardleys® or Tetramin®, which are enriched with vitamin C. The wet diet consists of a combination of mussel (white or black), pink prawn, and fish in a 1:1:1 ratio, which is minced together with some spinach or lettuce leaves in a blender. A general marine fish mineral and vitamin premix (available from BASF Vitamix Ltd. in Cape Town) is added to the food at a rate of 2 % by mass. The food is bound by adding 10 % by mass of gelatin to the mixture. The food is then placed into ice cube racks and frozen. The fish are fed mussel and wet food at 5% body weight per feeding, and flakes are sprinkled into the tanks *at libitum*. Pieces of lettuce leaf should be hung in the tanks as well. In the wild, 36 % of the clownfishes' natural diet is made up of vegetation.

6.3 Egg collection.

It is vital to know exactly when the eggs were laid, so that their hatching date can be anticipated. The eggs must be removed just prior to hatching; if they hatch in the brood stock tank it is very difficult to collect the larvae without damaging them, and if they are left in the tank the parents will feed on them. Egg development rate is species specific, and depends on the temperature of the water. It takes the eggs of most species of clownfish between 6 to 8 days to hatch at 26 °C. The development rate of the eggs of a healthy pair of fish in a stable environment should be constant, and can be monitored through colour and other changes. The eggs are removed from the brood stock tank in the late afternoon, about an hour before sunset on the eve of hatching. The fish should be encouraged to lay their eggs on a removable surface, such as a flowerpot refuge, or on a ceramic tile or false side of the tank (Fig. 16).

A small bucket is placed in the tank, and the egg-bearing surface is placed into it, and then gently lifted out of the tank. The eggs must be kept submerged in water at all times. The bucket is then re-submerged in the larval tank, and the egg-bearing surface is transferred to a cradle in the larval tank. The brood stock and larval tank water temperatures must be the same. The cradle can be constructed of any inert material. PVC pipe and fittings (20 mm) are used at the Rhodes University hatchery (Fig. 17).

Figure 16. *A Clarke's clownfish male guarding eggs laid on a removable tile.*

The eggs need to be constantly agitated by a gentle flow of water when in the larval tank. The constant movement prevents clumping and smothering, which makes the eggs susceptible to fungus and bacterial attack, and ensures that the eggs are well aerated. The parent fish usually perform this function by fanning the eggs with their pectoral fins. A gentle jet of recirculated larval tank water can be directed onto the eggs to perform this function, but the simplest approach is to place the eggs perpendicular to and within a gently rising stream of bubbles from an air stone.

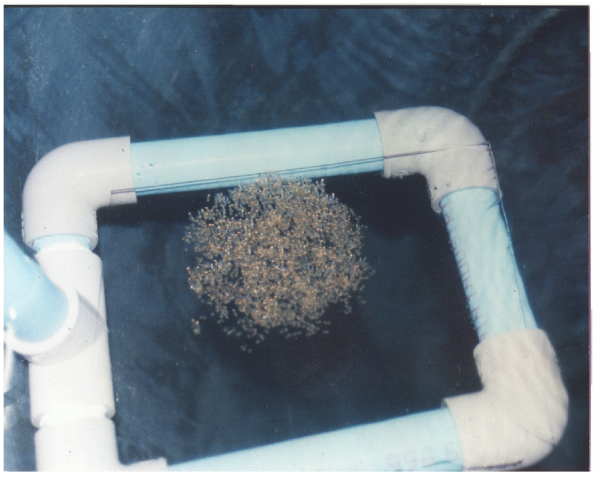


Figure 17. *An egg-bearing glass pane rests on a PVC cradle in the larval tank.*

The eggs are not lost if they are laid on the walls of the tank. A technique developed at the Rhodes University hatchery allows the hardy eggs to be cut off the walls of the tank using a sharp blade. As the eggs are freed from the side of the tank they are siphoned out of the tank into a bucket. The water in the bucket is then gently stirred, so that the eggs concentrate in the middle of the bucket. They are siphoned out of the bucket into a plastic cone (a rain gauge is an excellent substitute). A hollow glass tube is placed into the cone, and connected to an air supply. Just enough air is forced through the glass tube to produce a gentle stream of bubbles, which is vigorous enough to keep the negatively buoyant eggs in suspension. The cone is then floated in the larval tank, to ensure that the water temperature in the cone remains the same as that in the larval tank, until hatching has taken place.

Hatching occurs about 45 to 60 minutes after sunset, or lights out. The blue night-light is also extinguished for the duration of the hatching period. If a light is played on the eggs just prior to or during hatching, hatching will stop. It is therefore always a good policy to know exactly when the lights were turned out, and to give the eggs at least 1½ to 2 hours of darkness before checking to see if they have hatched. Hatching rates should be at least 80% of the egg batch. If the hatching rates are poor, or hatching occurs over two or more days, it is likely that the eggs are of poor quality. Poor quality eggs are the result of brood stock stress resulting from many factors, including disease, the environment and water quality. These kinds of factors normally result in eggs that deteriorate during development, which are removed from the batch by the parents before hatching. If an apparently healthy batch of eggs reaches full term, but has a poor hatch rate, it is most likely due to poor brood stock nutrition. Feeding live black mussel (*Perna perna*) ovaries to the brood stock pair usually boosts egg quality.

If the eggs have not hatched after two hours of darkness, it is safe to assume that they will not hatch that night. If they are attached to a movable surface they should be returned to the brood stock tank under the cover of darkness. The parents will continue to look after the eggs during the night and the following day. The eggs can be left in the larval tank, but the chances of fungal or bacterial infection are high. The eggs in the aerated cone can be left to gently tumble for at least another day, before hatching rates decline. At the Rhodes University hatchery, clownfish eggs have hatched after four days in a tumbling cone. Once the larvae have hatched, the cone is gently submerged into the larval tank. The

larvae are forced out of the cone by the airflow from the glass tube; and the cone is then reversed and removed from the tank.

The cradle and egg-bearing surface is removed from the larval tank once hatching has occurred, and the nightlight is turned on. At first the larvae appear disorientated, swimming in fits and jerks in all directions in the larval tank, but they should settle down after a few minutes. They will then begin to accumulate at the sides of the tank, at the reflective surfaces of the water and tank interface. Ideally, the larvae should be spread out evenly in the tank. This is accomplished by adding green water to the tank, to diffuse the light in the tank.

6.4 Larval rearing.

Green water consists of a purified monoculture of unicellular algae. Green water also buffers water quality changes in the larval tank, and provides a source of nutrition for rotifers, the first live organisms on which the larvae feed (See Live Food Culture). The algal culture is added to the larval tank until an optimum concentration is reached. There are billions of algal cells per millilitre in this solution, and the required number of algal cells varies depending on what species of algae is used. The correct amount of algal concentrate required in the tank is found by estimating the density of the cells in the concentrate. A Neubauer haemocytometer is used to count the algal cells in a number of 1.0 mℓ samples, and the results are averaged. The number of cells required per milliltre of larval tank water is then divided by the average number of cells per millilitre of algal concentrate, and multiplied by the volume of water in the larval tank. The result is the amount of algal concentrate that needs to be added to the larval tank. It is a time consuming process to carry out this procedure every time algae needs to be added to the larval tank. A less accurate but far simpler method of estimating algal density is by using turbidity. A clear plastic ruler with a white cord (or ribbon, elastic band, etc.) tied around one end is submerged in a larval tank containing the correct concentration of algae. The turbidity in the tank will cause the white band to fade away as it sinks into the depths of the tank. The amount of submerged ruler should be recorded at the point at which the band fades away. In future, the ruler can be submerged to this point, and then the concentrated algae is added until the band fades away once more.

After adding the algae, the water in the tank is gently aerated through an air

stone, but is not exchanged or recirculated. The larvae are then left alone until the following morning. The larvae do not require food immediately, as they initially obtain energy by absorbing their yolk sacs. The length of the yolk sac phase depends on the species and the individual larvae; the clownfish generally start feeding within 8 hours of hatching.

At first light the following morning the bottom of the larval tank is carefully siphoned clean of dead and damaged larvae and other debris. Healthy larvae should be swimming in the water column if the light conditions in the tank are correct. Fish lying on the bottom of the tank should be removed. Any organic matter on the bottom of the tank can lead to bacterial and fungal outbreaks. Enriched rotifers are then added to the larval tank, at a density of approximately 10 rotifers.mℓ^{-1} (See Live Food Culture for details). It is important that the correct amount of rotifers be added to the larval tanks. Too few rotifers mean that the larvae would not encounter enough of the rotifers to feed on. If there are too many rotifers, the larvae feed too much, forcing food through their rudimentary gut too quickly to be digested properly. Too many rotifers also have an adverse effect on water quality, using up too much oxygen and producing high levels of waste nitrogen metabolites. Feeding larvae remain relatively still in the water column. They take on a characteristic "S"-shape when feeding, darting forward to catch any prey which comes within range.

In a well-balanced larval tank environment, the rotifers should consume the algae, and the larvae will consume the rotifers. After 6 hours, the bottom of the tank is siphoned of debris, and the rotifer density in the tank is estimated by taking 1.0 mℓ water samples. The density of the algae is estimated by the turbidity method. The larval tank is then topped up with the required amount of enriched rotifers and algal concentrate.

The larval rearing room is on a 12L:12D photoperiod cycle. Thus the second six hour period ends with lights out. Just before lights out, the bottom of the larval tank is siphoned clean once more, and 25% of the water in the tank is drained out and slowly replaced by water from the larval recirculating filter system. Algal concentrate is added to the tank, and the drainage pipe of the tank is attached to a simple rotifer extraction filter system (Fig. 18). This airlift-driven system removes the leftover rotifers from the tank during the night. The rotifer filter system is removed at first light the next morning, and freshly enriched rotifers

and algae are added to the larval tank, as for the previous morning. This procedure is followed for 3 days with clownfish. On the fourth day the larvae are usually large enough to feed on *Artemia* nauplii.

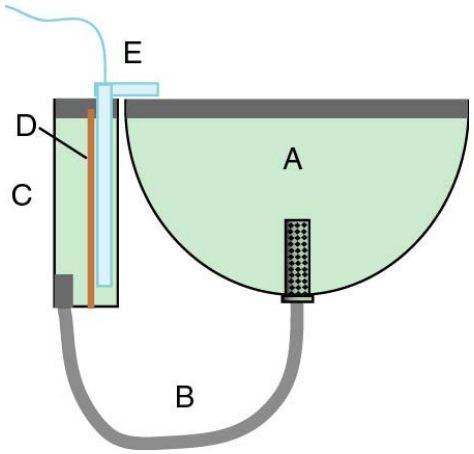


Figure 18. Rotifer extraction system connected to the larval tank (A) by the tank's drainage pipe (B). The system consists of a 200 mm PVC pipe (C) divided into two chambers by a 60 µm sieve (D). Inflowing water from the tank enters into one of the chambers, where the rotifers are concentrated, and the water is pumped back to the tank by an airlift (E) in the other chamber.

On the fourth day the drainage pipe of the tank is connected to the larval recirculating filter system, Algal concentrate is added to the tank in the usual way, but the enriched rotifers are only added to a density of 5 rotifers.mℓ^{-1}. Freshly hatched *Artemia* nauplii are also added to the tank, at a density of about 1 *Artemia*.mℓ^{-1}. The water inflow valve from the recirculating system is opened slightly, so that one full exchange of water occurs over six hours. The algal and live food densities are checked and replenished after six hours, as before. Just before lights out the 100 µm outflow sieve in the tank is replaced by a 350 µm sieve. The tank remains connected to the recirculating system overnight, and will be flushed clean of algae and live food by the morning. Only *Artemia* nauplii are fed to the larvae on the 5th day, at a density of 2 *Artemia*.mℓ^{-1}. Algae may be added if the larvae still show signs of concentrating at the sides of the tank. A donut-shaped cover is used to shade the sides of the tank at the Rhodes University hatchery, which is an effective way to keep the larvae away from the sides of the tank.

On the 6th day the clownfish larvae are fed on a combination of *Artemia* nauplii and

Instar 2+. *Artemia* reach the Instar 2 stage of development about 6 - 8 hours after hatching. Their digestive tract has developed during this time, and they are able to take up enrichment media such as Super Selco®, available from Inve Aquaculture Products, in Belgium. The *Artemia* nauplii and instar 2's are fed to the larvae twice per day, each at a density of about 1.0 *Artemia*.mℓ^{-1}. The larvae are fed on enriched *Artemia* only on the 7th day, at a density of about 1 *Artemia*.mℓ^{-1}.

The clownfish larvae begin to show the first signs of metamorphosis on the 8th day after hatching. Some of the larvae will begin to develop colouration similar to adult fish. Metamorphosis takes place from the 8th to the 14th day after hatching, depending on the species of clownfish, and larval quality. Clownfish larval survival to metamorphosis should be about 50 to 90 %, depending on species and hatchery worker experience. Post larval survival is usually at least 95 %. The larvae are weaned onto a dry, artificial diet after metamorphosis. Research at Rhodes University showed that larval weaning could be initiated as early as 7 days after hatching, with no reduction in survival rate. However, better larval growth rates were achieved if weaning occurred after metamorphosis. Small quantities of the fine particle food are sprinkled onto the surface of the tank during the day, while the larvae are still being fed on *Artemia*. After two to three days the post-larvae should be feeding on the artificial diet, and the *Artemia* can be discontinued. It is vital that the bottom of the tank is siphoned clean before each feeding, as the uneaten food that accumulates there is ideal for the proliferation of bacteria. Commercial larval weaning diets are available from Inve Aquaculture and other companies in Europe and the USA, in a range of particle sizes. Andrew Gordon[3] developed the larval weaning diet used at Rhodes University. His diet composition is shown in Table 3.

The post larvae are reared in the larval tank until they are robust enough to be moved out of the larval rearing room into the grow-out system. The clownfish post larvae can be moved any time between 15 and 45 days after metamorphosis, depending on the species. *Amphiprion clarkii*, for example, can be moved early as they are relatively hardy and grow quickly. *Amphiprion premnas* post larvae, on the other hand, are susceptible to shock, and can only be moved once they are more developed. During the post larval stage, the flow of water into the tanks is increased, so that one full exchange occurs every 1 to 2 hours. Crushed flake food

[3] Gordon, A. 1999. The effect of diet and age-at-weaning on growth and survival of clownfish *Amphiprion percula* (Pisces: Pomacentridae). MSc Thesis, Rhodes University, Grahamstown.

is introduced during this period.

Table 3. The composition of the larval weaning diet used at the Rhodes University marine aquarium fish hatchery.

Composition:	
Casein	35.0 %
Dextran	25.4 %
Fish meal	17.5 %
Gelatinised starch	10.0 %
Fish oil	6.0 %
Mineral premix	4.0 %
Vitamin premix	2.0 %
Protein value:	44.0 %
Fat content:	7.6 %
Ash content:	7.1 %
Moisture content:	8.3 %
Gross energy:	19.5 $mJ.kg^{-1}$

The juveniles are moved into the grow-out tanks when they are about 15 to 20 mm in length (Fig. 18). The water in the tank is drained to about 20 % of its capacity, and the larvae are gently scooped up out of the tank using a beaker or some other kind of vessel. They are *not* netted out of the tank. They are released into a bucket half full of larval tank water, which is floated in the grow-out tank. Grow-out tank water is added to the water in the bucket over the course of an hour, until the bucket sinks, releasing the juveniles into the grow-out tank.

6.5 Juvenile grow-out.

The juveniles are stocked into the grow-out tanks at a density of approximately one fish per litre (Fig. 19). They are fed on a commercially prepared dry diet at 6.0 - 8.0% of their body weight, twice per day. Commercial diets are available from various sources, such as Inve Aquaculture in Belgium. The dry diet can be supplemented with adult *Artemia,* flake food, pink prawn, mussel and the wet diet fed to the brood stock. The juvenile clownfish take about 2 to 4 months to reach market size, which is 30 – 40 mm total length. Fish with physical or colour

deformities are culled. Some of the best quality juveniles are kept until they reach maturity, and are used as brood stock.

Figure 19. *Amphiprion clarkii* juveniles stocked in the grow-out tanks at a density of about 1.0 fish.ℓ^{-1}.

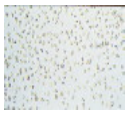
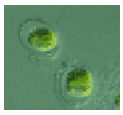
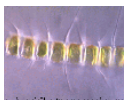
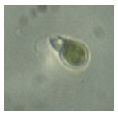
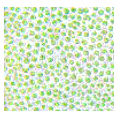
CHAPTER 7: LIVE FOOD CULTURE

One of the most difficult aspects of marine fish culture is the initiation of larval feeding. In the ocean, the larvae have access to a rich and abundant source of nutrition in the form of zooplankton and phytoplankton. Most marine fish larvae are very poorly developed upon hatching, and the food contained in their yolk sac is used up in a matter of hours. This means that they have to begin feeding almost immediately, at a very small size. Their digestive tract is very poorly developed, and can only absorb nutrients from ingested prey once it dies and begins to break down through autolysis. In other words, the larvae use the digestive enzymes of the prey to aid in the digestion of the prey. The enzymes are only active if the prey is alive at the time of ingestion. Also, the larvae only strike out at living prey, using their well-developed eyes to orientate on the moving organism. Ideally, live oceanic plankton should be the first food presented to the marine fish larvae on hatching. Unfortunately, it is impractical to harvest and keep plankton alive in a sustainable way at a land-based hatchery. For this reason, alternative live foods are used, such as the rotifer *Brachionus picatilis*, and the early stages of the brine shrimp *Artemia* sp. Most marine fish larvae would never encounter these organisms naturally, and they do not contain the nutrient content required by the larvae. The nutrient content of these organisms can be augmented by feeding them on a diet of micro-algae (phytoplankton) rich in the required nutrients, or by allowing them to take up artificial dietary supplements.

7.1 Micro-algae.

The micro-algae are a diverse group of unicellular organisms that may be sessile or motile, using cilia or flagella to move. They may have a simple cell wall, or one with cellulose like the higher plants, or a silicon-based exoskeleton (the diatoms). They also have different types of pigment, but they all have chlorophyll-containing chloroplasts in common. This means that they are all primary producers, converting light energy and chemicals into nutrients. Over 40 species of micro-algae have been used in aquaculture, but the most important species used in marine aquarium fish larviculture are presented in Table 4.

Table 4. Types of algae used in the larviculture of marine aquarium fish.

 ***Isochrysis***	Type: Size: EPA:DHA ratio: Temp: Salinity: Light Intensity:	Chrysophyta (Golden brown algae) 4 - 8 µm in diameter 0.5:9 % total fatty acids 25 - 30 °C 10 - 30 ‰ 2 500 - 10 000 Lux
 ***Nannochloropsis***	Type: Size: EPA:DHA ratio: Temp: Salinity: Light Intensity:	Chrysophyta (Golden brown algae) 4 - 6 µm in diameter 30.5:0 % total fatty acids 20 - 30 °C 0 - 36 ‰ 2 500 - 8000 Lux
 ***Tetraselmis***	*Type:* Size: EPA:DHA ratio: Temp: Salinity: Light Intensity:	Chlorophyta (Green algae) 12 - 14 µm in long axis 6.4:0 % total fatty acids 20 - 28 °C 20 - 40 ‰ 5 000 - 10 000 Lux
 ***Chaetoceros***	*Type:* Size: EPA:DHA ratio: Temp: Salinity: Light Intensity:	Chrysophyta (Diatoms) 15 -17 µ m in long axis 11:1 % total fatty acids 25 - 35 °C 20 - 35 ‰ 8 000 - 10 000 Lux
 ***Dunaliella***	*Type:* Size: EPA:DHA ratio: Temp: Salinity: Light Intensity:	Chlorophyta (Green algae) 7 - 12 µ m in long axis ? 12 - 35 °C 35 ‰ 3 000 – 10 000 Lux
 ***Chlorella***	Type: Size: EPA:DHA ratio: Temp: Salinity: Light Intensity:	Chlorophyta (Green algae) 2 - 10 µ in diameter ? 10 - 28 °C 26 - 30 ‰ 2 500 - 5 000 Lux

Nannochloropsis sp. is the phytoplankton cultured for green water at the Rhodes University marine hatchery. The species was chosen because it is euryhaline, and can be cultured in fresh water. This decreased the amount of seawater required by the hatchery (which had to be transported 45 km inland to the hatchery from the coast), and stopped the algal culture from being infected by rotifers, which cannot tolerate fresh water. However, the nutritional quality of the algae is dependent on the health of the algae. The shock of introducing freshwater-cultured algae into the full strength seawater of the larval tank had a negative impact on the quality of the algae, necessitating the use of seawater as the culture medium.

The algal culture process is initiated with a monoculture inoculant of the desired algal species. These inoculants are commercially available from biological supply companies worldwide. The more widely used marine algal species may be obtained from research centres, such as the Marine and Coastal Management's Sea Point laboratory in Cape Town. The inoculant is added to a 1.0 ℓ volumetric flask containing an algal growth medium. Commercially prepared media are available from biological supply companies, or can be made up from one of the many published recipes. The following recipe has been modified from that published by Rob Toonen, of the University of California (Table 5).

Table 5. Algal growth medium recipe.

Ingredient	Volume
Filtered, UV sterilised seawater	950 mℓ
Distilled freshwater	50 mℓ
1 M KNO_3	1 mℓ
12 mM KH_2PO_4	5 mℓ
+ Metals solution	1 mℓ
+ Vitamin solution	1 mℓ

The metals solution is made up from four stock solutions (Tables 6). The stock solutions can be stored in the fridge (at 4 - 6 °C) indefinitely.

Table 6. The stock solutions that combine to make up the Metals solution.

Stock solution 1:	1 ℓ of distilled water + 48 mg $ZnSO_47H_2O$ (or 30 mg of $ZnSO_4H_2O$) + 30 mg $CoSO_47H_2O$ + 3.0 g H_3B0_3
Stock solution 2:	1 ℓ of distilled water + 25 mg $Na_2MoO_42H_2O$
Stock solution 3:	100 mℓ distilled water + 5.0 g $FeC1_36H_2O$ +2.0 g $MnSO_4$ (Do not disturb precipitate)
Stock solution 4:	1 ℓ of distilled water + 50.0 g $Na_2EDTA2H_2O$
To combine Solutions:	850 mℓ distilled water + 10 mℓ stock solution 1 + 10 mℓ stock solution 2 + 10 mℓ stock solution 3 + 100 mℓ stock solution 4 + NAH until pH = 7.5 + Top-up the solution with distilled water until 1 ℓ

The vitamin stock solution can also be kept for a long time, if the container is wrapped in tin foil to keep out the light, and it is kept at a temperature below 4 °C. The solution consists of 0.5 mg cyanocobalamin (Vitamin B_{12}) + 0.025 mg Thiamine + 0.25 mg Biotin dissolved in 250 mℓ distilled water.

After the culture medium has been made up in the 1.0 ℓ volumetric flask, it is sterilised in a microwave oven for two minutes. Filtered air is then bubbled through the solution to remove carbon dioxide, and the flask is sealed with a bung. Once the solution has cooled to ambient temperature the algal inoculant is added to the flask. The flask is sealed once more with a bung that has two apertures. Filtered air is gently bubbled into the solution via an airline that passes through one of the apertures, and exhaust air passes through a filter connected to the other aperture. The solution in the flask must be kept sterile at all costs.

The flask is then stored in a temperature and light controlled cabinet. The back of the cabinet consists of a battery of vertically mounted full-spectrum neon light tubes. There should be enough neon tubes to provide a light intensity of about 10,000 Lux in the cabinet, for a period of 14 hours per day. The cabinet must be large enough to contain the 1.0 ℓ flasks as well as a number of 20 ℓ clear-glass "carboy" flasks. Under the correct light and temperature conditions it will take approximately 10 days for the algae in the culture solution to bloom to densities in excess of 10,000,000 cells.mℓ$^{-1}$. Once this occurs, two 1.0 ℓ flasks of algal culture are decanted into one of the carboy flasks, which contain 18 ℓ of UV light and microwave radiation-sterilised, filtered seawater. The carboy flask is sealed in the same way as the 1.0 ℓ flask, and air is bubbled into the new algal solution. Once the algal bloom has reached maximum density after about 10 days, the algae in the carboy flasks can be used to supply green water to the hatchery directly, or to inoculate small algal ponds, depending on the requirements of the hatchery. A series of maturing 1.0 ℓ and 20 ℓ algal solutions are maintained at the Rhodes University hatchery, to provide inoculant for two 400 ℓ circular, fibreglass algal tanks. The algal contents of two 20 ℓ carboys are used per tank. The tanks are each lit by a 650 W metal halide overhead lamp, and strongly aerated, to keep the algae in suspension. These tanks provide more than enough *Nannochloropsis* algae for the requirements of the hatchery.

7.2 Rotifers.

There are over 2,000 species of rotifers, ranging in size from 40 - 2000 μm. Most are freshwater species, but some occur in the marine environment. The most common rotifer used in marine larviculture is *Brachionus plicatilis*, a euryhaline species that measures about 130 - 200 μm in long axis. It can tolerate salinity as low as 15 ‰, but grows optimally at a salt concentration of 25 - 35 ‰. These

rotifers have two modes of reproduction; during adverse environmental conditions, they reproduce sexually, by producing "resting" eggs, or cysts. Male rotifers are only present during these conditions. They are much smaller than the females, have no feeding structures and only live for a day. The encysted eggs are very hardy and can be kept in storage for a long time.

During ideal conditions the females reproduce by producing delicate eggs, through parthenogenesis. One to two eggs are carried on the tail at a time (Fig. 20), and it takes about three days for a female to reach maturity. They live for about 10 days. Asexual reproduction is very fast, and is the preferred mode when culturing rotifers as live food.

Figure 20. A female rotifer carrying a single large egg.

Batch culture is the simplest and most common way to grow rotifers for hatchery consumption. Specific strains of small, high quality *Brachionus plicatilis* are available from biological supply companies as resting eggs. These eggs are hatched in a 1 ℓ flask of seawater containing a density of about 50 million algal cells.mℓ^{-1}, at a temperature of 20 - 25 °C. Ten days after hatching the algae should disappear and the rotifers will be ready to harvest. They are then stocked out into a 1.0 m^3 tank containing algae, at a stocking density of 10 rotifers.ℓ^{-1}. The tank is gently aerated, as the rotifers are weak swimmers and prefer still water. After 10 days the rotifer concentration should be about 100 000 rotifers.ℓ^{-1}. These rotifers can be harvested over a number of days if the algae is replenished each day, but

eventually the toxins in the tank will accumulate and cause the culture to crash. For this reason a number of rotifer tanks are maintained at different stages of development. Once a crash occurs, 80 % of the water is replaced and algae is added to the tank once more. There should be enough rotifers and resting eggs left in the tank to reseed it, and within 10 - 20 days the tank can be harvested again.

Wild rotifers will colonize static water bodies under the right conditions, and can be cultured and harvested in the above manner, if they are suitable for the larval species being cultured. Care must be taken to avoid contaminating the rotifer tanks with copepods. These organisms feed on rotifers, and they will decimate a rotifer population in a few days. They are difficult to eradicate once they have infected a culture system.

The rotifers are obligate feeders, capturing all particles of 2 - 20 μm in their vicinity in their buccal cilia and crushing them with their remarkable mastex mouthparts. This hammermill-like organ can crush just about any organic particle, allowing the rotifer to feed on a range of organic matter. The most common alternative food to algae that is used to culture rotifers is brewers, marine or torula yeast. The natural protein and fatty-acid content of the rotifers is usually not enough for marine larvae, but feeding the rotifers on substances rich in the required compounds can augment these levels. Algae are an excellent source of fatty acids, and yeast is high in protein, which is why they are often used as a combination feed. The problem with yeast is that it causes a rapid deterioration in water quality if allowed to accumulate and breakdown in the tank.

Commercial rotifer food is available, such as Culture Selco®, produced by Inve Aquaculture in Belgium. Culture Selco consists of enriched yeast cells, which have been manipulated for better digestion. The rotifers can also be enriched with specially developed enrichment diets, such as Protein Selco® (protein) and Super Selco® (fatty acids), also available from Inve Aquaculture. The directions for use of each of these products are clearly laid out on their labels. The rotifers are collected from the culture tanks using a fine net (< 100 μm). The rotifers are very delicate, and must be treated carefully. A concentrating device should be used instead of scooping the rotifers directly out of the rotifer tanks with the net. The simplest device consists of a lidded 10 ℓ bucket with a 20 mm hole drilled into it, about ¾ of the way up the side. Insert a short length of 20 mm PVC pipe into the

hole, to act as an overflow spout. Cut a hole in the lid of the bucket, so that the mouth of the net can rest on the lid, with the mesh hanging into the bucket. The bucket is placed above the rotifer tank, and a smaller vessel is used to gently pour water from the rotifer tank into the bucket, through the net. The rotifers collect in the partially submerged net, while the water overflows back into the rotifer tank. Once the rotifers have been concentrated they are washed, by displacing the water in the bucket with at least 20 ℓ of sterilized seawater. The concentrated rotifers are siphoned from the submerged net into another 10 ℓ bucket, and enriched according to the method described on the enrichment package label. This usually entails adding a calculated volume of an emulsified enrichment solution to the bucket, for a number of hours. The rotifers are then poured through the concentrating device again, and washed with at least 20 - 30 ℓ of sterilized seawater, as the enrichment solutions tend to make the rotifers very sticky, and can contaminate the larval tank water. Three 1.0 mℓ samples of the clean, enriched rotifers are then counted on a slide under a microscope, to estimate the number rotifers in the bucket. The correct volume of concentrated rotifers is gently siphoned into the tank through an aquarium air tube, to make up a density of 10 rotifers.mℓ^{-1} in the larval tanks.

7.3 *Artemia*

Artemia, or brine shrimp as they are more commonly known, are small crustaceans that are found in saltpans around the world. Like rotifers, they have two modes of reproduction. During adverse conditions of high salinity, chronic food shortages and/or cyclic oxygen stress (less than 2 mg.ℓ^{-1}) the *Artemia* produce cysts, which are encapsulated, desiccated embryos that are metabolically inactive (Fig. 21). The cysts can remain dormant for many years as long as they are kept dry and oxygen free. *Artemia* cysts are collected from saltpans and sold in a vacuum packed container, after being cleaned and dried. *Artemia* can be ordered directly from the producers, biological supply companies or aquarium hobbyist shops. Once the package has been opened, the quality of the cysts deteriorates over time. Tightly resealing the package after the air in it has been displaced by pure Nitrogen gas, and keeping it in the refrigerator can ameliorate this deterioration to some extent. When the cysts are re-hydrated the embryos resume their development.

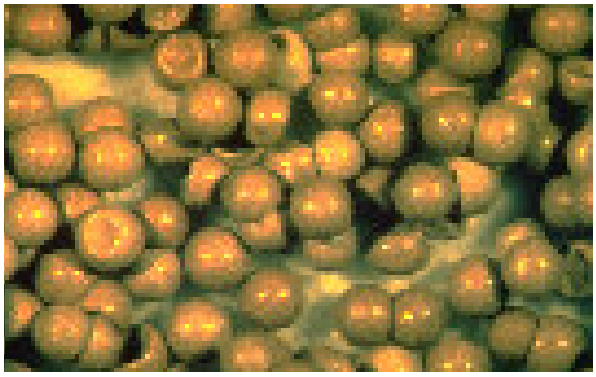


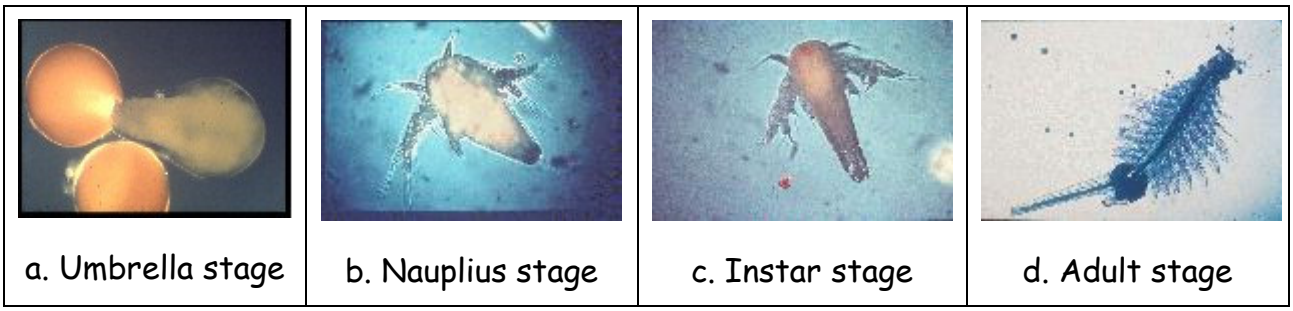
Figure 21. Encapsulated, dehydrated *Artemia* cysts.

The shell should be removed from the cysts before they are hatched. This is accomplished by first re-hydrating the cysts in seawater for 60 to 90 minutes, at 25 °C. The cysts are re-hydrated in a 1.0 - 2.0 ℓ,"V"-shaped *Artemia* hatching vessel. A rain gauge is ideal for this purpose, but a 2.0 ℓ plastic soft-drink bottle with the bottom removed and the cap in place can also be used. One to 10 grams of *Artemia* cysts are added per litre of seawater, and the mixture is vigorously aerated through a hollow glass rod (or straw) that extends to the bottom of the hatching vessel. This arrangement ensures that the cysts are aerated and kept in suspension. After re-hydration the cysts are poured out of the vessel into a tubular sieve. The sieve consists of a 50 mm slice of 110 mm PVC pipe, with a 100 - 150 μm mesh glued over the lower end. The shell consists mainly of chitin, and can be dissolved with a weak (10 - 12 %) hypochlorite solution, as found in household bleaching products such as Jik®. A shallow bowl is filled with the solution and the cysts resting on the mesh of the sieve are submerged in it. The bowl is then placed on a magnetic stirrer, and the cysts are stirred in the bleach for about 3 minutes. The cysts can also be stirred manually, using a spatula or teaspoon. De-encapsulation time will vary, depending on the thickness of the shell and the bleach concentration. It is best to estimate when de-encapsulation is complete by observing the colour changes that occur during the process. An encapsulated cyst is darkish brown in colour, but as the shell dissolves it changes to a lighter brown or greyish colour. The fully exposed embryo is orange in colour. Once the majority of the cysts appear to be orange, the sieve is removed from the bleach and washed gently under a cold-water tap with municipal water, until the cysts no longer smell of chlorine. If the cysts are not washed enough after the shell has been removed, the bleach will destroy the embryos. The de-encapsulated *Artemia* cysts are then washed off the sieve into a clean hatching vessel with sterile seawater. The vessel is topped up with sterile seawater, the glass rod bubbler is replaced and the

hatching vessel is placed into a controlled temperature water bath, set to 25 °C. The water bath can be simply constructed from and old glass aquarium, with a thermostat-controlled aquarium heater as the heat source. The hatching vessels should be constantly illuminated with a light intensity of about 2000 Lux.

After 18 - 20 hours at 25 °C the cyst bursts and the embryo is exposed. The cost of *Artemia* cysts varies, depending on the quality of the strain, and availability. *Artemia* quality is a function of the small size of the nauplii, their nutritional value and their hatching success. Good quality cysts will have a hatch rate of more than 90 %, meaning that 200 000 to 300 000 nauplii will hatch per gram of cysts.

For the first few hours, the embryo hangs beneath the cyst, still enclosed in a hatching membrane (Fig. 22a). This is called the Umbrella stage, during which the embryo completes its development and emerges as a free-swimming nauplius (Fig. 22b). The newly hatched nauplii do not feed externally because their mouth and anus are not fully developed, but survive by absorbing their yolk. Approximately 6 to 12 hours after hatching they moult into the second larval stage, or Instar, and begin to feed by filtering organic particles from the water column (Fig. 22c). The nauplii will grow and progress through 10 Instars and 4 sub-adult moults before reaching adulthood, in about 8 days (Fig. 22d).



a. Umbrella stage	b. Nauplius stage	c. Instar stage	d. Adult stage

Figure 22. The primary development stages of *Artemia* sp…

The *Artemia* are most nutritious to the fish larvae during the nauplius stage, due to the presence of yolk. The nauplii of certain strains of *Artemia* are so small that they can also be used as a first feed for some species of larval fish (eg. The AF240 strain, available from Inve Aquaculture). The nauplii stage can be prolonged for several hours by placing them into water cooled to about 6 °C. The fish larvae can then be fed for the whole day from one batch of nauplii. To accomplish this, the nauplii are stored in a refrigerator in their hatching vessels. Air-lines are inserted into the refrigerator to keep the water in the vessels bubbling.

Artemia are not part of the natural diet of marine larvae, and often need to be nutritionally enriched. The *Artemia* are obligate feeders, scooping up particles from the water column with hairs on their swimming appendages, and passing the food parcels up into the mouth in a continuous wave. Non-nutritious particles (even glass beads!) pass directly through the digestive tract, while nutritious particles are absorbed. *Artemia* are thus ideal bio-encapsulators; they absorb enrichment diets as well as transport some of the enrichment into the larvae, in their guts. The *Artemia* are enriched in the same way as rotifers, by exposing them to high concentrations of highly unsaturated fatty acid (HUFA) solutions such as Inve Aquaculture's Super Selco® before feeding. As with the rotifers, the *Artemia* need to be carefully washed clean of the Super Selco® before being added to the larval tanks.

Under optimal environmental and feed conditions, fertilized female *Artemia* usually produce ovoviviparous, free swimming nauplii at a rate of up to 75 nauplii per day. They will produce 10 to 11 broods over an average life cycle of 50 days. Under perfect conditions, an adult *Artemia* can live as long as three months and produce up to 300 nauplii every 4 days. Adult *Artemia* average about 8 mm long, but can reach lengths of up to 20 mm under the right conditions. An adult grows 20 times longer and 500 times heavier than the nauplii stage. The *Artemia* are a nutritious and readily eaten food source for marine fish of all ages. It is always a good idea to have a stock of adult *Artemia* available, for sensitive, difficult-to-feed or convalescing juvenile fish, or adult fish that are being conditioned for breeding.

The optimal temperature for adult *Artemia* grow-out is from 25 - 30 °C, but there are differences between strains. For example, the San Francisco bay strain prefers 22 °C, compared to 30 °C for Great Salt Lake *Artemia*. Brine Shrimp prefer a salinity of 30-35 ‰, a pH of around 8 and high oxygen levels. The *Artemia* should have a pale pink or yellow-orange appearance. If they are feeding on micro-algae they may look greenish in colour. Under ideal conditions growth and reproduction is rapid, and a self-sustaining *Artemia* supply is possible. If they are red in colour, they are experiencing oxygen stress, and are producing haemoglobin. If they continue to be stressed, they will start producing resting cysts, and the colony will crash. It is important to have a vigorous air supply in the tank for two reasons; to keep the available food supply in suspension where it can be filtered out; and to promote a good oxygen supply in the system.

The adult *Artemia* can be reared at high densities in recirculating systems, but expensive, high-tech equipment is required. At the Rhodes University hatchery,

they are grown out in batch cultures, in a series of vinyl children's paddling pools or Port-a-pools® contained within a small agricultural tunnel (Fig. 23). The *Artemia* feed on micro-algae such as *Nannochloropsis*, or the softer yeast cells like Torula yeast, as well as on a multitude of other organic matter, which is colonised by bacteria, from which the *Artemia* gain sustenance.

Figure 23. A small agricultural tunnel is used to grow-out *Artemia* biomass at the Rhodes University marine hatchery.

CHAPTER 8: SYMPTOMS, DISEASE DIAGNOSIS AND TREATMENT.

Although most aquarists aspire to keeping disease-free aquariums, it unlikely that such a state can be achieved for any length of time, particularly in large, commercial systems. It has even been argued that a truly disease-free environment would be deleterious to the fish; they would lose their natural immunity to disease, and on leaving this environment they would be defenceless. In the wild, the fish's immune system and low population density limit the occurrence of disease epidemics. In aquariums and commercial recirculating systems the small water volume and relatively high stocking densities allow for devastating disease outbreaks.

Imagine that fish and disease[4] co-exist in equilibrium in the aquarium system, where the immune system of the fish defends it against infection. If the immune systems of just a few of the fish are compromised and they can no longer resist infection, the balance is disturbed. The disease proliferates until a critical point is reached where even healthy fish succumb to infection. Immune system depression is a symptom of *stress*, and all outbreaks of infectious disease can be traced to stressed fish. Fish are easily stressed, through transport, handling, abrupt changes in environment (eg. temperature, salinity, light intensity), poor water quality, density, aggression, toxins, food quality, lack of refuge and unsuitable substrate, to name a few possibilities.

When a fish experiences stress, hormones are released which change its metabolic state from anabolic (energy is taken up and stored) to catabolic (stored energy is rapidly used up). In the wild, this state only lasts for a short duration. After the cause of the stress has been avoided the fish is able to rest and recuperate. If the stress lasts for more than a few minutes, the fish becomes exhausted. A side effect of the stress hormone cortisol is that it causes a decrease in the number of white blood cells in the blood. The white blood cells are a vertebrate's primary internal form of defence, attacking and consuming foreign cells such as bacteria, as well as producing antibodies to fight viral infections and parasites. The combination of exhaustion and diminished white blood cell numbers leaves the fish highly vulnerable to infection. Depending on the incubation time of the disease, the after-effects of stress can take two to four weeks to be manifest.

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[4]Used as a generic term for all invasives, including parasites and toxicants.

Stress avoidance through careful management is the single most powerful tool that the aquarist or fish farmer has in the fight against disease. Careful management can effectively eliminate disease epidemics by providing and maintaining a stress-free environment for the fish. The requirements of the fish must be well understood and constantly monitored. If fish need to be handled or transported, it may be a good idea to use anaesthetics such as 2-phenoxyethanol. Any fish showing unusual behaviour or signs of stress should be immediately removed from the culture system and placed into a quarantine system for observation, and possible treatment.

The fish in a culture facility must be kept under constant observation for physical or behavioural indications of stress and infection. By the time a fish begins to show signs of distress it will already be infected, so the aquarist must be prepared to make an immediate diagnosis and act accordingly. The only way to be sure which disease the fish has is to kill it and immediately carry out an autopsy. This may be possible where many fish are available and one can be sacrificed, but is not practical when rare, expensive or brood stock fish are sick. Usually, aquarists have to treat their fish on the basis of the symptoms displayed by the fish. The symptoms of the most common diseases of captive marine tropical fish are well documented, and are presented in Table 6.

Carefully note all the symptoms displayed by the sick fish, and check them off against the symptoms listed in Table 6. Add up the coloured squares and read off that number at the top of the table that corresponds to the colour of the most squares. Use this number in Table 7 to find out which disease causes the symptoms, and how to treat the disease. The most common diseases of captive marine tropical fish are velvet, marine ich, *Vibrio*, fish TB and fin rot. A short summary of the diseases listed in Table 7 follows. A more accurate means of identifying the pathogen is included in the summary.

Table 6. Symptoms which may be experienced by captive marine tropical fish, and their possible causes.

Possible cause	1	2	3	4	5	6	7	8	9	10	11	12
Change in colour												
Cloudy eyes												
Distended gut												
Erratic swimming												
Emaciation												
Fin erosion												
Rapid breathing												
Gill anaemia												
Loss of appetite												
Listless												
Pop-eye												
Skin haemorrhage												
Rough skin												
Flashing												
Ulcers												
White patches												
White spots												
Warts												

Table 7. The common diseases and treatments of captive marine aquarium fish.

Possible cause	Disease	Treatment
1	Velvet	- Freshwater bath - Copper treatment
2	Marine Ich	- Formalin bath - Copper treatment
3	Vibrio	- Antibiotics - Adjust water quality
4	Fin rot	- Antibiotics - Adjust water quality
5	Fish TB	- Combined antibiotic therapy (ie. doxycycline + rifampin)
6	Clownfish disease	- Copper treatment - Adjust water quality
7	Fungus	- No treatment - Good hygiene, sterilize food
8	Microsporidian	- No treatment - Euthanasia
9	Lymphocystis	- No chemical treatment - Adjust water quality
10	Fish lice	- Physical removal of parasite - Freshwater or formalin bath
11	Flukes	- Freshwater bath - Formalin bath
12	Poisoning	- Serial water change - Remove fish to new system

Velvet

a.k.a: Oodinium, marine velvet, coral velvet

Species: *Amyloodinium ocellateum*

Type: Dinoflagellate protozoan.

Mode: Mobile dinospores attack fish, infesting gills and skin; become non-motile parasitic trophonts which attach to epithelium using deep-penetrating rhizoids; encyst and drop off host, becoming reproductive palmella stage, which can then release 256 active dinospores after 3 days, or remain dormant for up to a month until conditions become favourable.

Identification: Kill fish by pithing. Take a clean microscope slide and slide it along the posterior half of the fish, including the caudal fin. Cover the slide with a coverslip. Remove a gill arch from the fish and smear the gill epithelium along the slide. Cover with a coverslip. View slides under a compound microscope at a magnification of 100 - 200x. The encysting trophonts will be easily identifiable as dark, circular nodes attached to the epithelium of the fish. The cysts range from 20 - 80 μm in diameter.

Marine Ich

a.k.a: White spot, salt water ich, cryptocaryon

Species: *Cryptocaryon irritans*

Type: Holotrichous ciliate protozoan

Mode: Mobile tomite stage actively seeks out host, attaching to the epithelium of fish. Becomes parasitic trophont that vigorously burrows into the epithelium using a rasping collar of teeth-like cilia. The trophonts enlarge and drop off the host, adhering to

the substrate of the tank, encysting within 24 hours to form the reproductive tomont stage. After 6 days, up to 200 tomites are released.

Identification: Anaesthetise fish. Place fish under a dissecting microscope. Observe fins and flanks of the fish. The trophonts will be clearly perceived as whitish, oval nodules on the surface of the skin. The trophonts range from about 50 - 450 μm in long axis.

Vibrio

a.k.a: Wipe out, gas gut disease

Species: *Vibrio septicaemia*

Type: Haemorrhagic septicaemia bacteria

Mode: Ubiquitous in marine water. It is most likely a secondary invader, attacking fish weakened by another disease. Grows in swarming colonies in the host.

Identification: Pith fish and open up the abdominal cavity. The liver, swimbladder, peritoneum, intestine and gall bladder will appear reddish and swollen. The gills will appear anaemic. External lesions are visible. Positive identification of the bacteria will require cultures to be made and tested at a laboratory.

Fin rot

a.k.a: Pseudomonas, red spot disease, ulcer disease

Species: *Pseudomonas fluorescens*

Type: Haemorrhagic septicaemia bacteria

Mode: Ubiquitous in all water bodies. Usually a secondary invader of damaged tissue, but can also be a primary pathogen in stressed

fish.

Identification: Fin and tail erosion is the primary external indicator for this disease. An autopsy will reveal bruising due to capillary rupture on the surface of the pericardium, intestine, heart, kidneys, liver and gills. Positive identification of the bacteria will require cultures to be made and tested at a laboratory.

Fish TB

a.k.a: Piscine tuberculosis, acid-fast disease, granuloma disease

Species: *Mycobacterium marinum*

Type: Chronic progressive disease-inducing bacteria

Mode: Ubiquitous in all water bodies. The mode of infection is uncertain, probably through damaged epithelium and by ingestion of contaminated food.

Identification: An autopsy under a dissecting microscope will reveal greyish-white nodules in the liver, kidney, heart and spleen. Positive identification of the bacteria will require cultures to be made and tested at a laboratory.

Clownfish disease

a.k.a: Brooklynella, Angelfish disease

Species: *Brooklynella hostilis*

Type: Ciliate protozoan

Mode: Present in all marine water bodies. Heavy infestations almost entirely due to parasite:host equilibrium being disrupted by stress through poor water quality. Parasite can multiply very quickly through asexual reproduction.

Identification: Kill fish by pithing. Take a clean microscope slide and slide it along the posterior half of the fish, including the caudal fin. Cover the slide with a coverslip. Remove a gill arch from the fish and smear the gill epithelium along the slide. Cover with a coverslip. View slides under a compound microscope at a magnification of 100 - 200x. The parasites will appear as mobile kidney-shaped cells, surrounded by an indistinct corona indicating cilia. They are about 50 - 80 μm in long axis.

Fungus

a.k.a: Traummelkrankheit, reeling disease, sandpaper disease

Species: *Ichthyophonus hoferi*

Type: Marine fungus

Mode: The fungus most likely enters the fish through food infected with resting spores. The spores germinate in the fish, forming hyphae that invade heavily vascularized tissue such as the heart, liver, spleen, kidney, and lateral body muscles. Daughter spores are usually produced asexually in the hyphae, but can also be produced in the resting spore, without hyphae. Sexual reproduction may take place if two hyphae meld.

Identification: An autopsy under a dissecting microscope will reveal greyish-white nodules in the liver, kidney, heart, spleen and muscle tissue; very similar in appearance to fish TB pathology. Positive identification will require cultures to be made and tested at a laboratory.

Microsporidian

a.k.a: Beko disease

Species: *Microsporidium* spp.

Type: Microsporidian protozoa

Mode: May be associated with rotifers and *Artemia* as intermediate hosts. Fish infected through oral ingestion of spores. Spore injects sporoplasm into a host cell through a hollow tube, which then divides mitotically to produce masses of cells. Colonises intestinal, bile, liver, lymphatic, muscle, neural, subcutaneous, and gonadal tissue.

Identification: An autopsy under a dissecting microscope will reveal a white, rigid, thickened intestinal wall, and cysts within the gut.

Lymphocytsis

a.k.a: Viral disease, cauliflower disease

Species: *Lymphocystivirus* spp.

Type: Irido-virus

Mode: Occurs globally. Nothing is known about the means of infection. Normally found in fish experiencing behavioural or environmental stress. Not usually fatal, should go into remission once stress alleviated.

Identification: External tumour-like growths, containing pearl-like nodules, when viewed under a dissecting microscope; normally white, but can range in colour from cream to brown. No internal indications.

Fish lice

a.k.a: copepods, isopods

Species: Generic term for many species

Type: Mobile parasitic copepods, parasitic isopods

Mode: Both groups have representative species in marine water bodies throughout the world. They are mobile and actively seek out hosts, where they attach to the epithelium of the fish and feed. The copepods abrade the skin of the fish and may cause serious lesions. The isopods usually attach to the gills, inside the mouth or on the skin where they can feed on the blood of the host.

Identification: "Fish lice" are relatively large (3.0 mm - 12.0 mm) and can be identified with the naked eye. However, they will drop off a dying host. On post mortem, one should look for eroded gill filaments, usually in one gill chamber only; lesions on the skin surface under the scales, and in the case of severe copepod infestation, loosening and sloughing off of the skin in the anterior region of the fish.

Flukes

a.k.a: Trematodes

Species: Generic term for many species

Type: Digenetic trematode

Mode: Usually have a complex life cycle of which fish are only one of a few hosts, including mollusks, crustaceans and even birds; thus rare in established marine aquarium systems; mostly found in newly acquired wild-caught fish. The migrating larvae of the flukes, called the cercariae, move from one host and actively seek out the next. Once they find a fish they can follow chemical, mechanical and sometimes visual cues to invade specific tissues, including muscle, skin and eyes. The adult flukes are normally found in the digestive tract of a fish, but can also infest the gallbladder, circulation system and abdominal cavity.

Identification: Use a dissecting microscope to examine the abdominal cavity and intestine of a freshly dead fish. You should observe the presence of small, leaf-shaped worms in the tissue.

Treatments

A few standard treatments can be used for a number of diseases, as was shown in Table 7. A detailed description of the methods and treatments used at the Rhodes University marine aquarium fish hatchery follows.

Copper

Prepare a 1.0 $g.\ell^{-1}$ stock solution of copper citrate solution by dissolving 6.0 g copper sulphate ($CuSO_4$) and 4.0 g citric acid into 1.5 ℓ tap water. The solution must be stored in a cool, dark place, and should be stirred vigorously before use.

Remove as much calcium or magnesium carbonate-rich material from the system as possible (eg. coral, shells, dolomite chips). Also remove *all* invertebrates from the system (eg. anemones, soft coral, cleaner shrimps, hermit crabs).

Add an initial dose of 30 mℓ copper citrate solution per 100 ℓ seawater to the system. After the initial dose, add 15 mℓ copper citrate solution to every 100 ℓ of water exchanged in the system, over a period of 4 weeks.

After 4 weeks, the copper treatment can be discontinued. The copper solution can be diluted and removed in time, through water top-ups and exchange. However, if invertebrates are to be returned to the system, a 100 % water change is required.

Freshwater bath

Fill approximately $2/3^{rds}$ of a dark-coloured bucket with freshwater from the tap. Float the bucket in the tank of the fish to be treated, until the water temperature in the bucket is

the same as in the tank.

While the temperatures are equalising, bubble air through the water in the bucket to extract excess chlorine. Once the water temperatures are the same, remove the infected fish from the tank using a net, and place it gently into the bucket. Cover the bucket, as the fish will react vigorously to the freshwater. Treat the fish for 5 minutes, and then return it to the tank. Repeat treatment daily for 5 days.

Formalin bath

Remove seawater from the infected tank in a darkly coloured bucket. Immediately add 2 mℓ of 37 % formaldehyde per litre of water in the bucket.

Remove the fish from the tank and place it in the bucket. Gently bubble air into the bucket through an air stone, and place a cover over the bucket to calm the fish, to stop it jumping out. If the ambient temperature is low, float the bucket in the tank so that the temperature in the bucket remains constant. Do not allow any of the formalin-treated water to spill into the tank!

After an hour, return the fish to the tank. Repeat the treatment daily for 5 days.

Antibiotics

<u>Doxycycline and rifampicin combination</u>

Fish TB is highly resistant to general antibiotic treatment, although some success has been had with various combinations of antibiotics. The most successful combination reported by Stoskopf[5] was doxycycline and rifampicin. The antibiotics are administered orally in the food of the infected fish. Doxycycline and rifampicin must be administered to the fish daily, at doses of 2 $mg.kg^{-1}$ and 10 $mg.kg^{-1}$ body weight respectively, for 14 days.

[5] Stoskopf. M.K. 1993. Fish Medicine. WB Saunders & Co.

Oxytetracycline

While there are many other antibiotics available for the treatment of bacterial infections in fish, oxytetracycline is relatively cheap and easy to obtain, covers a broad spectrum of diseases, and is stable in seawater. Unlike other tetracyclines, oxytetracycline does not have a critical effect on the nitrifying biological filter. The antibiotic is still active after being frozen or heated to 100 °C, which makes it ideal for incorporation into fish food. Antibiotics can be administered to the fish in the following ways:

Bath: This is the least effective means of treating the fish, and should be used for external infections only.

Seawater is removed from the infected tank in a dark bucket. Oxytetracycline is added to the bucket at a concentration of 400 mg per litre of seawater. The solution must be vigorously stirred, until the oxytetracycline is completely dissolved in the water.

The infected fish is placed into the bucket, and the solution is aerated. The bucket must be covered to calm the fish, to stop the break down of oxytetracycline through photodecomposition, and to stop the fish from jumping.

The fish is returned to the tank after one hour, and the treatment is repeated daily for seven days.

Oral: The preferred method of administering antibiotics to the fish; unfortunately one of the first signs of bacterial infection in fish is a loss of appetite.

The infected fish need to consume 60 mg of oxytetracycline per kilogram of fish body weight per day, until the infection has disappeared. The daily food consumption of the fish has to be

estimated, and the oxytetracycline incorporated into the food accordingly. The antibiotic can be added to the wet diet, or flake food can be soaked in dissolved oxytetracycline and then dried. As the quantities of oxytetracycline required are so small, it is usually a good idea to made up a large batch of treatment food, and store this in the freezer until needed.

A new approach to the oral administration of antibiotics is through bio-encapsulation, using live *Artemia*. The *Artemia* are continuous feeders, and if they are placed in a solution of oxytetracycline they will take up some of the antibiotic. These antibiotic-rich *Artemia* are then fed to the infected fish. This technique holds great promise, as the dose can be regulated by varying the concentration of the antibiotic in the water, or by changing the *Artemia* exposure time. Although some success has been experienced using this technique at Rhodes University, it needs to be researched further.

Injection

Injecting fish with antibiotics insures that the fish receive the correct dose, but it can be highly stressful for fish as small as most marine aquarium fish. It is a good idea to anaesthetise the fish first. The infected fish require a dosage of 0.1 mg oxytetracycline per 10.0 g body weight per day. To achieve this low concentration the oxytetracycline must be diluted with saline solution (7.0 g NaCl.ℓ^{-1} distilled water). The amount of solution injected into the fish should not exceed 1.0 $\mu\ell.g^{-1}$ body weight. Ideally, a microlitre syringe should be used.

Prepare a stock solution by dissolving 10.0 mg oxytetracycline and 7.0 g NaCl in 1.0 ℓ distilled water. Do not expose the solution to sunlight, and keep refrigerated.

Anaesthetise the infected fish in 0.2 mℓ 2-phenoxyethanol dissolved in 1.0 ℓ seawater. The fish will lose orientation quickly, but may take a while to be fully anaesthetised. Once the fish no longer responds to external stimuli (tapping of the bucket, or gentle prodding) it can be removed, blotted with an absorbent

towel and injected.

Inject 1.0 $\mu\ell.g^{-1}$ body weight oxytetracycline stock solution into the dorsal musculature of the fish, below the dorsal spine, approximately midway between the lateral line and the dorsal fin. Take care not to damage the fish's scales; insert the needle beneath a scale, in the direction of the head. Inject the solution into the fish carefully, and then place a thumb over the insertion point and slowly withdraw the needle, so that the thumb seals the hole as the needle is removed.

Return the fish to the tank and observe that it recovers from the anaesthetic. Repeat the process daily, until the fish has recovered.

Serial water exchange

If the fish in a system are showing symptoms of intoxication or poisoning, the first step is to find the source of the toxin and remove it. Ideally one could then change all the water in the system to remove the dissolved toxin from the system. Unfortunately such a radical water change would further stress the fish. It is more prudent to carry out a water change over a series of days, never exchanging more than 50 % of the water per day. The problem with serial water changes is that the toxin is diluted, but not completely removed. It takes five days to remove more than 95 % of the toxin from the system (Table 8).

If the source of the toxin cannot be traced, or the fish are showing chronic symptoms of intoxication and do not react to a 50 % water exchange, the fish must be removed and placed into a *mature* aquarium in the quarantine system, and kept under observation for secondary infections. Mature aquaria are tanks in which the correct water quality for the fish is maintained, and the biological filtration beds are fully developed, but the fish have not yet been added.

Table 8. An example of the effect that a serial water exchange has on the concentration of a toxin.

Day	Toxin concentration	Water exchange	Total toxin removed
1	10.0 mg.ℓ^{-1}	50 %	50 %
2	5.0 mg.ℓ^{-1}	50 %	75 %
3	2.5 mg.ℓ^{-1}	50 %	87.5 %
4	1.3 mg.ℓ^{-1}	50 %	93.8 %
5	0.8 mg.ℓ^{-1}	50 %	96.9 %